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약학석사 학위논문

Part 1. 분자모델링을 이용한 항암활성을 갖는 1,3,4-oxadiazin-5(*6H*)-one 유도체 약물 설계

Part 1. Design of the 1,3,4-oxadiazin-5(6*H*)-one derivatives with antitumor activities using molecular modeling

Part 2. C6 위치의 작용기를 가진 1,3,4-oxadiazin-5(*6H*)-one 유도체 합성 및 치환기 효과에 대한 연구

Part 2. Study on synthesis and substituent effects of 1,3,4-oxadiazin-5(*6H*)-one derivatives functionalized at the C6 position

2017 년 2 월

서울대학교 대학원

약학과 약품제조화학 전공

박 승 건

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Part 2. Study on synthesis and substituent effects of 1,3,4-oxadiazin-5(*6H*)-one derivatives functionalized at the C6 position

지도교수 이 지 연

이 논문을 약학석사 학위논문으로 제출함

2017 년 2 월

서울대학교 대학원

약학과 약품제조화학 전공

박 승 건

박 승 건 의 석사학위논문을 인준함

2017 년 2 월

위 원 장 _____ (인)

부 위 원 장 _____ (인)

위 원 _____ (인)

초 록

[Part 1]

1,3,4-oxadiazin-5(6*H*)-one은 N과 O를 포함하는 heterocyclic 골격인 peptide-mimetic 화합물이다. 1,3,4-oxadiazin-5(6*H*)-one의 N4와 C2 위치의 유도체에 대해 많은 연구가 진행되고 있으며, 항암효과와 신경치료효과를 포함한 생리활성을 가지고 있다고 보고된 바 있다. 최근에 본 실험실에서는 1,3,4-oxadiazin-5(6*H*)-one의 C6 위치에 작용기를 첨가한 유도체가 항암활성을 나타낸다는 연구를 보고하였다.

본 연구에서는 1,3,4-oxadiazinone 골격의 C6 위치의 작용기에 따른 물질의 생리활성을 파악하는 것을 목표로 하였고, C6 위치에 알킬그룹과 방향족 그룹을 도입한 다양한 1,3,4-oxadiazin-5(6*H*)-one 유도체를 합성하였다. MTT assay을 이용하여 1,3,4-oxadiazinone의 활성을 스크리닝하고, 분자 모델링을 이용하여 보다 다양한 치환기를 도입한 화합물을 설계하였다. 본 연구에서 탐구한 전략을 통해 N과 O를 포함하는 heterocyclic 골격의 작용기를 확장하여 개선된 활성을 나타내는 새로운 물질을 개발하는 방향을 제시할 수 있을 것이다.

주요어: 1,3,4-oxadiazin-5(6*H*)-one, 작용기, 분자 모델링

학번: 2015-21875

[Part 2]

1,3,4-oxadiazin-5(6*H*)-one은 N과 O를 포함하는 heterocyclic 골격 화합물로, 항암효과와 신경치료효과가 있다고 보고되었다. 1,3,4-oxadiazin-5(6*H*)-one의 N4와 C2 위치의 유도체에 대해 많은 연구가 진행되고 있다. 최근에 본 실험실에서는 1,3,4-oxadiazin-5(6*H*)-one의 C6 위치에 작용기를 첨가한 유도체가 항암활성을 나타낸다는 연구를 보고하였다.

본 연구에서는 1,3,4-oxadiazin-5(6*H*)-one 골격의 작용기를 확장하는 것을 목표로 하였고, C6 위치에 파라(para)자리가 치환된 페닐기를 보유하는 새로운 1,3,4-

oxadiazin-5(6*H*)-one 유도체를 합성하였다. Arylhydrazide의 EDC 커플링과 염기를 이용한 고리화 반응을 통해 1,3,4-oxadiazin-5(6*H*)-one 유도체를 높은 수율(50-90%)로 얻을 수 있었다. 고리화 반응은 C6에 위치하는 파라자리에 위치하는 페닐기의 치환기의 전자효과에 따라 영향을 받는 결과를 보였다. 본 연구에서 탐구한 합성 전략은 N과 O를 포함하는 heterocyclic 골격의 작용기를 확장하여 활성을 나타내는 새로운 물질을 개발할 수 있는 다양한 도구로 사용될 것이다.

주요어: 1,3,4-oxadiazin-5(6*H*)-one, 고리화반응, 치환기, 전자효과

학번: 2015-21875

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Part 1.

분자모델링을 이용한 항암활성을 갖는 1,3,4-oxadiazin-5(*6H*)-one 유도체 약물 설계

1. 서론

종양은 전세계에서 발생하는 주요 사망의 원인으로, 미국에서 발생하는 사망원인으로 두번째로 많이 발생하는 질환이다[1-2]. 2014년에는 종양판정을 받은 1450만명의 미국인들이 있으며, 2016년에만 미국에서 약 170만건의 새로운 종양이 진단되고, 약 60만명의 미국인이 사망한다.[3] 남성에서는 전립선암, 폐암, 직장 및 대장암, 방광암, 췌장암, 간암이 주를 이루며, 여성에서는 유방암, 폐암, 직장 및 대장암, 췌장암, 난소암이 주를 이룬다. [1-2]

항암요법은 1940년에 전립선암을 치료하기 위한 호르몬 요법으로 처음 시도가 되었다.[4] 항암치료를 위해 주로 사용되는 약물로는 항암성 알킬화제, 대사저해제, microtubule 저해제, topoisomerase 저해제, 세포독성 항생제 등이 있다. 대부분 약물은 종양세포의 성장을 억제하거나, ROS를 생성하거나 DNA와 결합을 일으켜 직접 사멸을 유도하는 작용기전으로 항암활성을 나타낸다.[5] 항암치료 요법으로는 항암제를 단일로 사용하는 단일제제 항암요법과 복합제제 항암요법이 있으며, 복합제제 항암요법에서 항암내성종양의 발생을 줄이고 부작용 면에서 보다 단일제제 항암요법보다 개선되었다.[6-7] 항암요법은 대부분 정상세포와 항암세포의 세포분화속도의 차이에 기반을 두기 때문에, 부작용이 다양하게 나타난다. 대표적인 부작용으로 오심, 구토, 탈모, 피로, 식욕저하, 성기능장애, 조혈세포의 기능이상 등이 있다.[4, 8]

타겟-항암요법이 1990년대에 알려졌다. 이는 정상세포와 종양세포의 신호전달과정, 혈관신생작용 및 화학구조와 생물학적 구조의 차이를 이용한 것이다. 이 방법은 종양세포에 주로 작용을 하고, 정상세포에는 작용이 적기 때문에 부작용을 줄일 수 있다.[4] 신호전달과정 경로에서 단백질 키나아제의 인산화는 중요한 기전으로, 종양세포의 생존과 증식에 매우 중요한 역할을 한다.[9] 신호전달경로 항암요법에서 대표적인 약물은 백혈병치료제인 Bcr-Abl 키나아제 저해제인 imatinib (상품명 글리벡)이 있다. 혈관신생저해제는 혈관내피세포성장인자(VEGF)와 혈관내피세포성장인자 수용체(VEGFR) 신호경로를 저해하여 혈관신생을 억제하고, 종양세포가 증식 후 혈관을 통해 전이되는 것을 막아준다.[4]

IGF1R(인슐린 유사 성장인자 타입 I 수용체, insulin-like growth factor-type I receptor)은 IGF-1을 주요 기질로 하는 tyrosine kinase receptor의 일종이다.[10] IGF1R은 2개의 세포 밖 α-subunit이, 세포질 티로신 키나아제 활성을 가지고 있는 2개의 transmembrane-spanning β-subunit에 disulfide 결합을 하고 있다 (Figure 1).[11]

IGF1R의 신호경로는 PDGF, EGF와 IGF 등을 비롯한 여러 성장인자들의 복합적인 작용에 의해 발생한다. IGF1R은 세포성장, 증식, 분화에서 중요한 역할을 하며, 특히 IGF 신호는 근육, 연골, 뼈의 형성과 심근과 뇌기능의 유지 등과 같은 생리학적으로도 중요한 역할을 하기 때문에 신생아, 청소년기 성장에 중요하다.[12-14] IGF1R의 주요 기능은 세포증식을 촉진하며, 프로그램 된 세포자살을 억제하는 역할을 한다. 유방암, 췌장암, 난소암 등을 포함한 다양한 종양세포에서는 IGF1R의 활성이 증가하는 신호이상을 나타낸다. IGF1R의 신호이상이 발생하게 되면, 성장인자 신호경로의 활성이 증가하게 되는데, 이는 세포증식, 전이 및 침습작용을 나타내는 종양세포의 발생을 유발하게 된다.[12-13, 15-16] 종양세포의 생존은 부분적으로 IGF1R의 항세포자살작용에 부분적으로 관련이 있다. 또한 IGF1R의 신호경로는 항암제에 대한 저항성의 증가와도 관련이 있다.[12] Src는 비수용체 단백질 tyrosine kinase로, IGF1R의 직접적인 인산화와 관계가 있다. Src은 신호전달체계에 작용하여, 종양세포의 생존력과 항암치료에 대한 저항성과 관계가 있다. 또한 Src의 활성 증가는 항암치료에서의 IGF1R 저해제에 대한 저항성과 관련이 있기 때문에[17-20] IGF1R과 Src의 저해는 매우 효과적인 항암치료의 전략이 될 것이다.

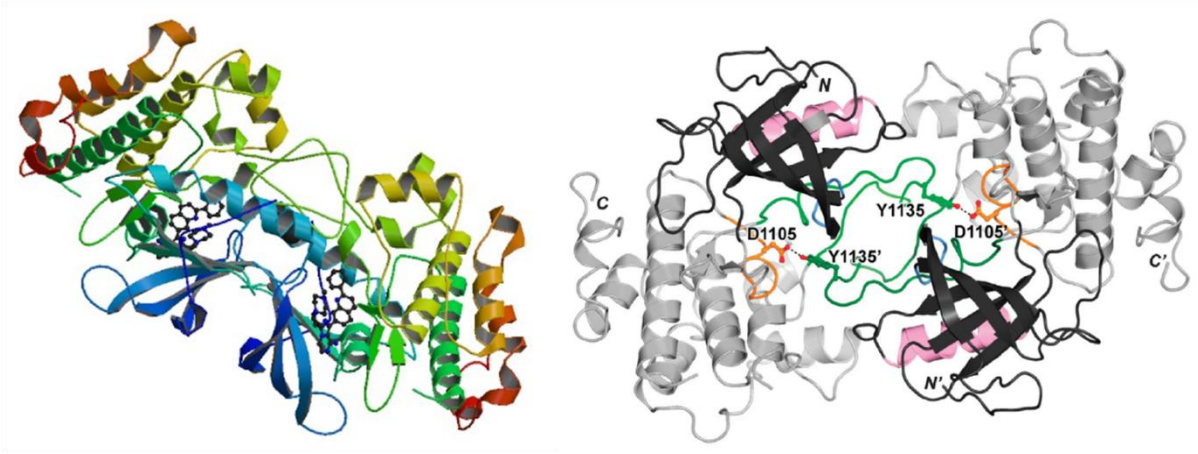


Figure 2. IGF1R의 크리스탈 구조

1,3,4-oxadiazin-5(*6H*)-one은 단단한 고리형태를 띠고 있는 peptoid 형태의 질소를 보유하는 헤테로고리 화합물로 solid phase peptoid synthesis (SPPS)에 의해 합성 할 수 있다.[21-22] 이 화합물은 항알츠하이머, 항종양, 항바이러스 및 항세균 작용과 같은 다양한 생리활성을 나타내는 것으로 보고되었으며[23-25] 최근에는 monoamine oxidase 저해제로 연구가 많이 이루어지고 있다.[24] 1,3,4-oxadiazin-5(*6H*)-one에 대한 합성과 생리활성에 관한 C2와 N4 위치에 관한 연구는 많이 보고되었으나[6, 21, 23, 26-33], C6 위치에 관한 연구는 거의 보고되지 않았다.[34] 따라서 본 실험실에서는 새로운 헤테로 고리를 보유하는 scaffold로서 1,3,4-oxadizin-5(*6H*)-one의 생리활성에 주목하였고, N4와 C6 위치의 functionalization을 통하여 항암활성에 대하여 IGF-1R과 Src를 타겟으로 한 실험을 진행을 하였고, 항암활성이 나타나는 효과가 나타났다.[22] 당시 본 실험실에서는 C6 위치에 치환기를 도입을 한 실험 또한 함께 진행하였지만, 당시에는 C6 위치에 Methyl, chloride, Phenyl기와 같은 간단한 작용기를 도입하였다. 이번 연구에서는 MTT assay와 함께 분자 모델링을 이용하여 보다 C6 위치에 대해 다양한 작용기를 보유한 화합물들이 수용체 결합자리에서 어떻게 결합을 하며, 작용을 하는지 확인할 수 있었다. 특히 분자 모델링을 통해 시각화하여 결합자리에 대한 화합물의 채움과 포즈를 확인할 수 있을 것이다. 이는 기존 연구보다 C6 위치의 작용기가 항암활성에서 개선된 효과를 나타낼 수 있는 방향을 제시할 수 있을 것이다

2. 본론

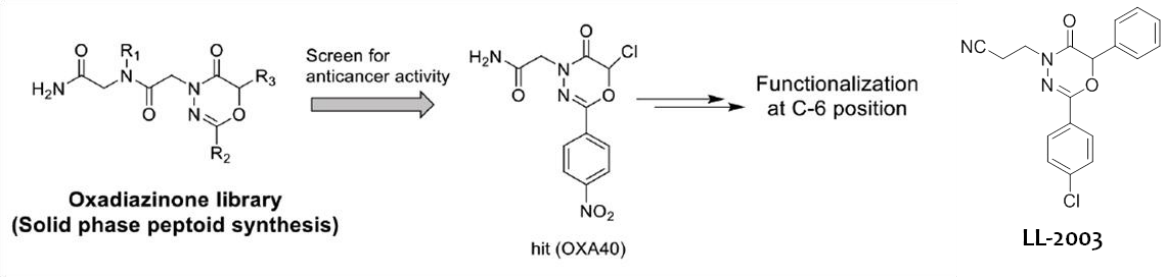
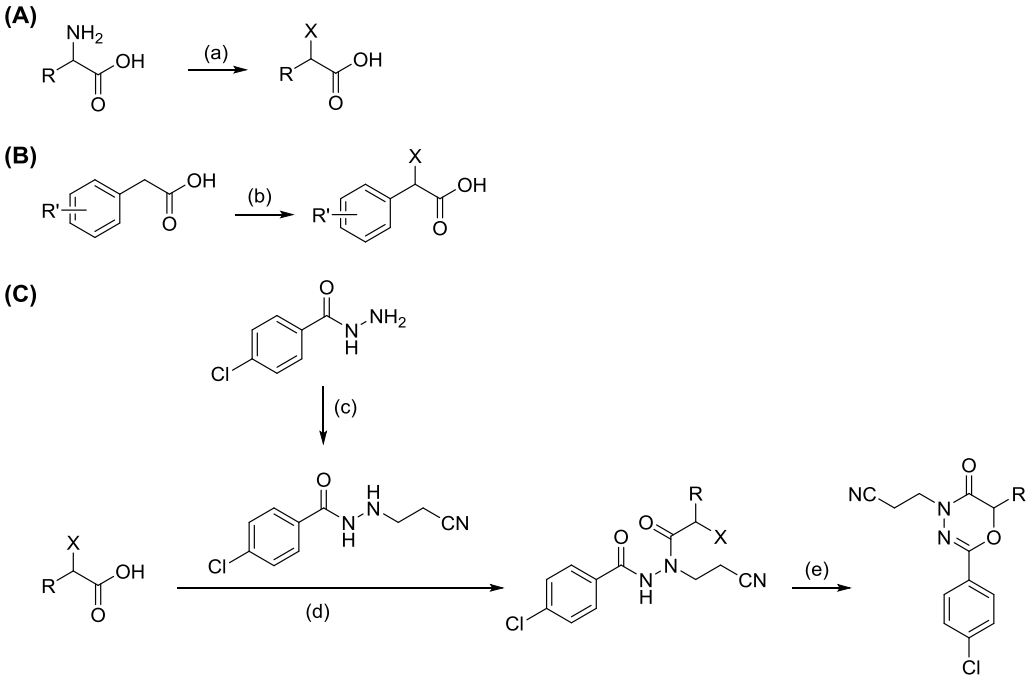


Figure 3. oxadiazinone library을 이용하여 C6 위치의 작용기를 도입한 화합물

본 실험실에서는 solid phase peptoid synthesis 방법으로 합성하여 구축한 1,3,4-oxadiazin-5(*6H*)-one의 library에서 C6 위치에 fuctionalization을 통해 새로운 화합물을 디자인하였다 (Figure 2).[22] 이번 연구에서는 C6 위치에 존재하는 작용기에 따라 항암활성이 존재하는지 또는 개선될 여지가 있는지를 확인하기 위하여 다양한 화합물을 다음과 같은 Scheme 1에 따라 합성하였다. Table 1은 도입한 작용기와 그에 대응하는 화합물을 나타낸 것이다.

2.1. 합성 전략



Scheme 1. C6 위치의 여러 작용기를 도입한 1,3,4-oxadiazin-5(*6H*)-one의 합성방법

Reagents and condition: (a) KBr (1 eq.), $NaNO_2$ (1 eq.), 2.5N H_2SO_4 (aq.), 2 hr; In case of tyrosine, $NaNO_2$ (1 eq.), 2.5N HCl, 2 hr (b) NBS (1 eq.), $(PhCO)_2$ (0.05 eq.), Benzene, reflux, overnight (c) Acrylonitrile (2.5 eq.), EtOH, reflux, 72 hr (d) EDC·HCl (1.5 eq.), CH_2Cl_2, 1 hr (e) K_2CO_3, CH_3CN, 50℃, 3 hr

Scheme 1의 (A)와 같이 아미노산에서 출발하는 화합물의 경우, KBr과 $NaNO_2$, 그리고 2.5N의 황산을 이용하여, 아미노기를 브롬으로 치환할 수 있었다. 그러나 tyrosine에서 출발하는 경우에는 $NaNO_2$와 2.5N HCl을 이용하여 아미노기를 염소로 치환할 수 있었다. 그리고 (B)와 같이 arylacetic acid에서 출발하는 화합물의 경우, NBS와 $(PhCO)_2$를 이용하여 α자리의 브롬을 치환을 진행하여 α-bromoarylacetic acid를 합성할 수 있었다. 4-chlorobenzohydrazide에 acrylonitrile을 이용한 alkylation을 진행한 (c) 과정을 통한 화합물을 합성하였다. (A) 과정을 통해 합성된 halogen이 도입된 산화합물을 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide을 이용한 EDC 커플링 반응을 진행한 후, K_2CO_3를 이용한 고리화 반응을 통하여 Table 1에 나타난 다양한 작용기를 도입한 화합물들을 합성할 수 있었다.

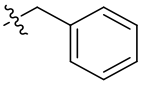
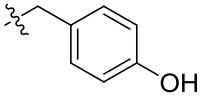
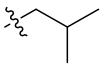
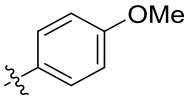
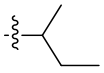
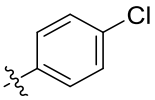
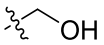
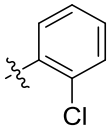
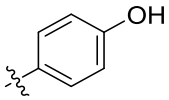
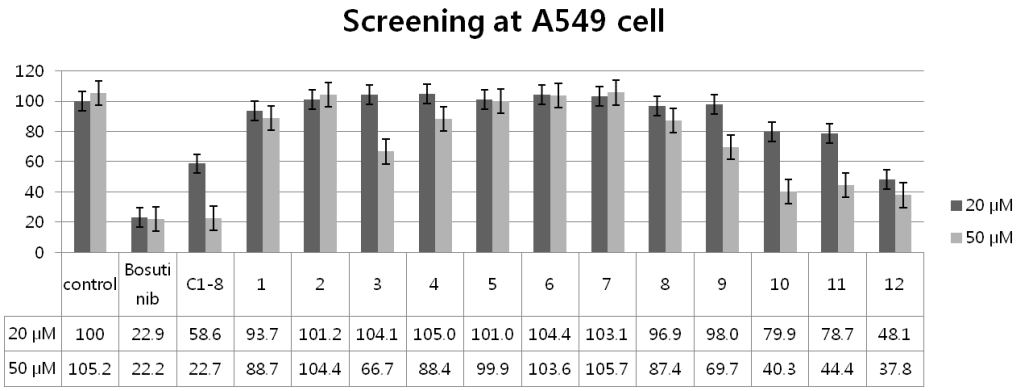
No.	R	No.	R
1		7	
2		8	
3		9	
4		10	
5		11	
6		12	

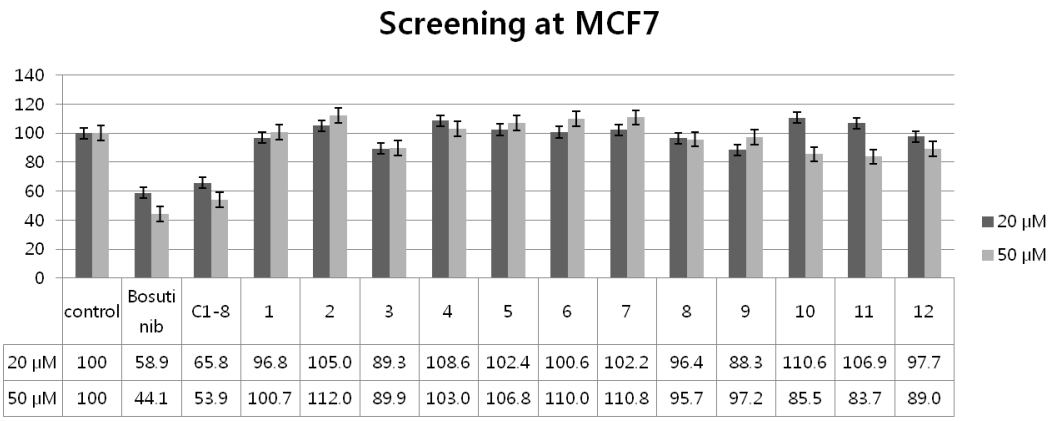
Table 1. 1,3,4-oxadiazin-5(*6H*)-one의 C6 위치에 도입한 화합물과 대응하는 작용기

2.2. MTT assay를 이용한 화합물의 항암활성 스크리닝

Scheme 1을 통하여 합성한 Table 1에 나타난 화합물의 항암활성효과를 확인하기 위한 실험으로 MTT assay를 진행을 하였다. 사람의 암세포의 일종인 A549와 MCF7 세포를 사용하였고, 각각의 세포마다 화합물을 20μm과 50μm의 농도로 3번씩 스크리닝을 진행을 하였다.



	control	Bosutinib	C1-8	1	2	3	4	5	6	7	8	9	10	11	12
20 μM	100	22.9	58.6	93.7	101.2	104.1	105.0	101.0	104.4	103.1	96.9	98.0	79.9	78.7	48.1
50 μM	105.2	22.2	22.7	88.7	104.4	66.7	88.4	99.9	103.6	105.7	87.4	69.7	40.3	44.4	37.8



	control	Bosutinib	C1-8	1	2	3	4	5	6	7	8	9	10	11	12
20 μM	100	58.9	65.8	96.8	105.0	89.3	108.6	102.4	100.6	102.2	96.4	88.3	110.6	106.9	97.7
50 μM	100	44.1	53.9	100.7	112.0	89.9	103.0	106.8	110.0	110.8	95.7	97.2	85.5	83.7	89.0

Figure 4. A549와 MCF7에 MTT Assay를 통한 화합물 스크리닝

다음 Figure 3는 암세포 중 하나인 A549와 MCF7 세포에 화합물을 처리하여 얻어낸 MTT assay 결과다. MCF7 세포에 대해서는 효과가 거의 나타나지 않았으나 A549 세포에 대하여 10~12의 경우 효과가 나타난 것으로 확인되었다. C6 위치에 치환기로써 alkyl그룹이 도입이 된 1~6은 활성이 미미하였으며, 페닐기가 도입이 된 7~12 중, C6 위치에 페닐기가 직접적으로 연결이 되지 않은 7~8은 활성이 미미하였다. 그러나

페닐기가 직접적으로 연결이 된 10~12가 활성이 크게 나타났다.

2.3. 모델링을 통한 화합물들의 결합 포즈 확인

앞선 MTT assay를 통해서 IGF1R에 대하여 화합물들이 활성을 나타냈고, 알맞은 IGF1R 수용체를 이용하여 분자모델링을 통한 화합물들의 결합과 포즈를 확인한 실험을 진행하였다. 화합물의 수용체에 대한 도킹을 진행하기 위해 사용한 수용체 단백질인 *Homo sapiens*의 IGF1R로 RCSB Protein Data Bank의 PDB 3D94을 선택하였으며, reference ligand로서, 3D94에 내재되어 있는 PQIP를 reference 물질로 이용하였다 (Figure 4). 사용한 프로그램은 Sybyl-X 2.1.1이며, docking simulation은 Sulflex-Dock Geom으로 진행하였다. 불러온 IGF1R 단백질인 3D94는 energy minimization으로 아미노산 말단에 존재하는 C와 N에 전하를 부여하였으며, 수소결합을 추가하였다. Charge를 부여하는 방식으로 Amber7 FF99 및 Gasteiger-Marsili을 선택하였다. Sidechain에 존재하는 amide는 fix를 시켰으며, Powell 방법을 이용하였다. Gradient는 0.001Kcal/mol*A와 Max iteraction은 10000의 값을 부여하였다. minimization을 진행하였다. 다음으로 ATP 결합자리에 존재하는 H_2O 1354를 제외하고 모두 제거하였고, Tripos의 force와 use current의 charge 방식으로 modify를 진행하였다. Docking을 마친 후, visualizer로써 BIOVIA 사의 discovery studio ver.4.5을 이용하여 시각화를 진행하였다.

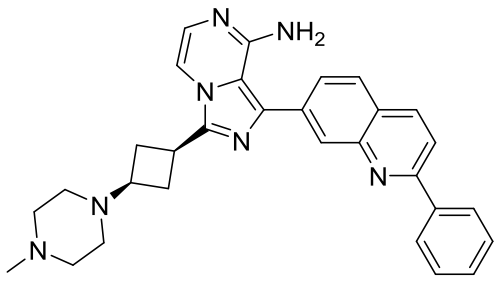


Figure 5. PQIP의 구조: cis-3-[3-(4-methyl-piperazin-l-yl)-cyclobutyl]-1-(2-phenyl-quinolin-7-yl)-imidazo[1,5-a]pyrazin-8-ylamine.

3D94의 내재리간드인 PQIP는 IGF1R의 ATP 결합자리에 결합을 하고 있다. C lobe의 활성루프 잔기인 Gly1122 그리고 Asp1123 잔기와, N lobe의 Met1049, Met1024와 Val1033 사이에 quinolinyl 치환기가 위치하여 반데르발스 힘으로 상호작용을 하고 있으며, Lys1003과는 수소결합을 한다. 특히 Met1049는 문지기(gatekeeper) 잔기의 역할을 한다. 또한 퀴놀린기에 연결된 페닐 치환기는 Phe980, Phe1124, Gly1125, Phe1017와 Ala1021은 반데르발스 힘으로 상호작용을 이루고 있다.[11] Table 1과 같이 합성된 화합물은 ATP 결합자리에 결합을 하게 된다 (Figure 5). LL-2003의 결합자리에 결합한 것과 비교했을 때, C6의 페닐기가 위치한 자리에는 알킬기가 존재하는 경우보다, 부피가 큰 아릴기를 가지고 있는 화합물의 경우에 보다 비슷한 자세로 결합이 이루어졌다. 결과적으로 C6 위치에 페닐기가 직접 연결이 된 화합물이 LL-2003과 비교했을 때, 보다 비슷한 형태로 IGF1R의 ATP 자리에 결합이 되었다.

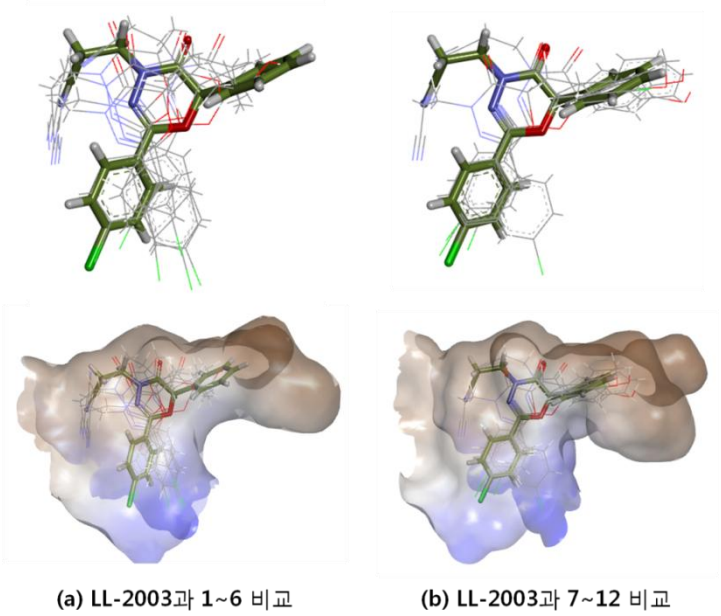


Figure 6. IGF1R에 LL-2003과 화합물 1~12의 도킹 결과

Name	Total_Score	Crash	Polar	Similarity
1	5.5488	-1.0234	1.1384	0.459
2	6.8448	-0.9576	1.006	0.517
3	6.4728	-0.9465	0.494	0.496
4	6.2864	-0.8759	1.4621	0.456
5	5.0902	-0.453	2.6116	0.414
6	5.2977	-1.4342	1.7235	0.506
7	7.3117	-1.7067	0.9792	0.511
8	7.111	-0.8283	2.2083	0.494
9	7.212	-0.779	0.9473	0.571
10	6.2784	-0.4769	1.7118	0.426
11	6.7101	-1.6164	1.2095	0.461
12	7.247	-1.1376	1.3021	0.459

Table 2. LL-2003과 합성한 화합물들의 Docking 결과

Modeling 결과에 따르면 C6 위치에 직접적으로 알킬기를 작용기로 가질 때보다 아릴기일 때 대체로 LL-2003과 비슷한 자세로 결합자리에 결합이 잘 이루어지는 것으로 나타났다. IGF1R에 대해 LL-2003이 효과가 있게 나타났으며, **Figure 3**에 의하면, MTT assay에 의하면 C6 위치에 페닐기가 직접 연결되어 도입이 된 물질에서 효과가 크게 나타났다. MTT assay에서 활성을 나타냈던 화합물들과 LL-2003이 어떤 IGF1R의 아미노산 잔기들과 상호작용을 이루는지 살펴보았다 **(Figure 6).** 4개의 화합물의 1,3,4-oxadiazin-5(*6H*)-one의 C6 위치에 존재하는 페닐기 부분이 모두 C lobe의 활성루프 잔기인 Gly1122 그리고 Asp1123 잔기와 N lobe의 문지기(gatekeeper) 잔기인 Met1049와, Met1024 그리고 Val1033 사이에 존재하여 반데르발스 힘에 의한 공통적인 결합력을 보이고 있다. 5Å 거리에서는 상호작용을 Gly1055와 수소결합을 보이고 있다. 즉 C lobe의 활성루프와 N lobe의 Met1049, Met1024와 Val1033 사이에 화합물이 존재하여 반데르발스 힘이 작용하는 것은 활성을 나타내는데 중요하다고 볼 수 있었다. 그러나 Phe980, Phe1124, Gly1125, Phe1017와 Ala1021 등과의 반데르발스 힘에 의한 상호작용이 거의 이루어지지 않았다. 따라서 C6 위치에 존재하는 부분에 대해 IGF1R의 비어있는 포켓을 더 채워주면 화합물들이 IGF1R 포켓 내에 존재하는 다른

아미노산들과의 결합을 더욱 증가시킬 수 있을 것이며, 이는 IGF1R 수용체에 대한 활성을 개선할 수 있을 것이라고 가정할 수 있었다.

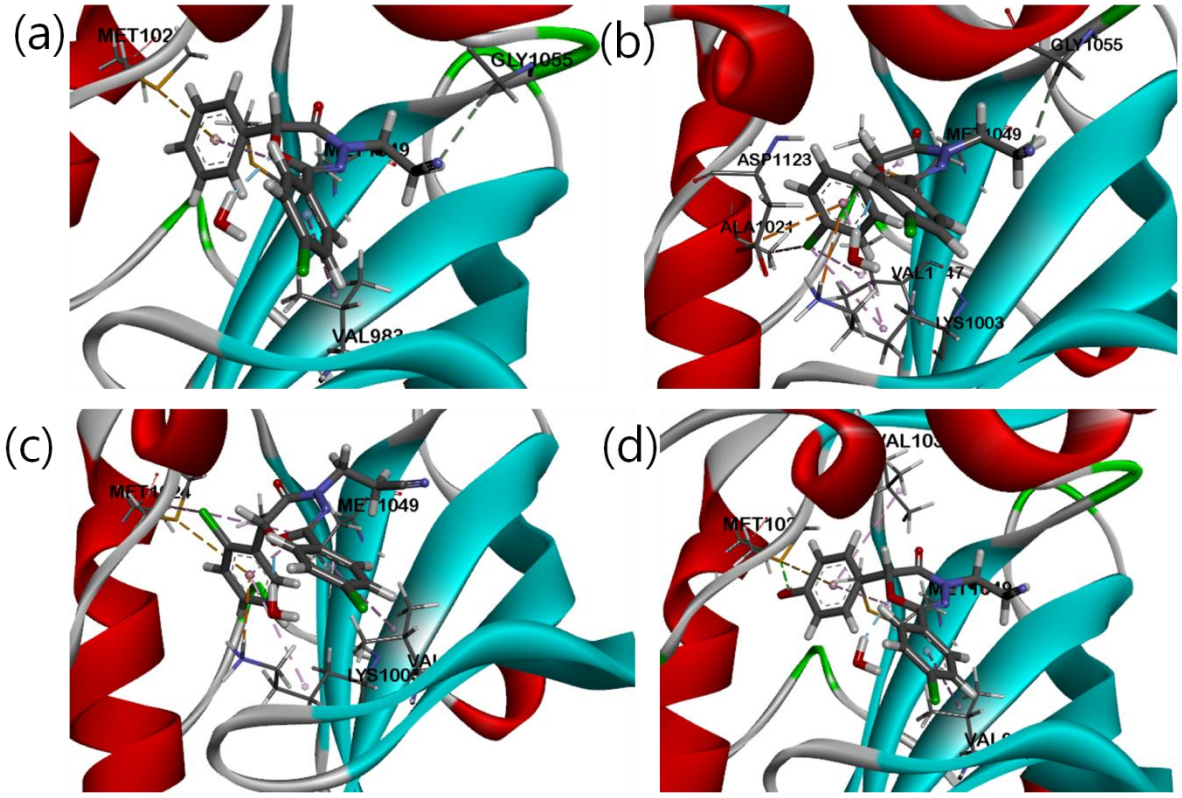


Figure 7. PDB3D94에 도킹한 후, IGF1R과 화합물들과의 상호작용을 나타내는 아미노산 및 크리스탈 구조. 각각에 대한 도킹한 화합물: (a) LL-2003, (b) **10**, (c) **11**, (d) **12**.

2.4. 새로운 아릴기를 가지는 화합물의 합성 및 모델링 분석

LL-2003보다 C6 위치에 존재하는 페닐기보다 부피가 큰 아릴기를 가지는 다양한 작용기를 도입한 물질들을 추가적으로 디자인하고 합성하였다 (Table 3). Scheme 1에서 사용한 동일한 방법으로, 다음 작용기가 도입된 화합물들을 합성 할 수 있었다.

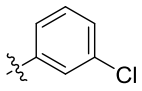
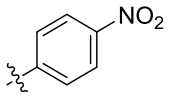
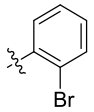
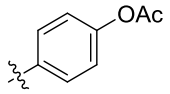
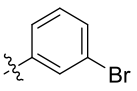
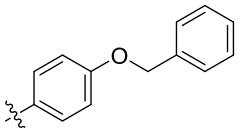
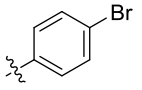
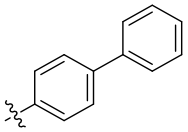
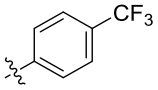
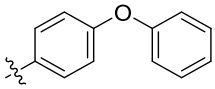
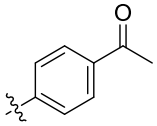
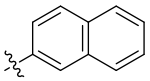
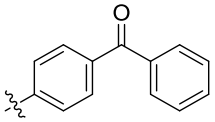
No.	X	No.	X
13		20	
14		21	
15		22	
16		23	
17		24	
18		25	
19			

Table 3. LL-2003의 C6 페닐기에 다양한 치환기가 도입이 된 1,3,4-oxadiazin-5(*6H*)-one 화합물과 작용기의 종류

Table 3의 화합물들을 IGF1R에 대하여 결합된 포즈를 비교하기 위하여, IGF1R (PDB 3D94)에 분자모델링을 이용한 도킹을 진행을 하였다. IGF1R에 대한 도킹결과는 Figure 7과 Table 4으로 나타났다.

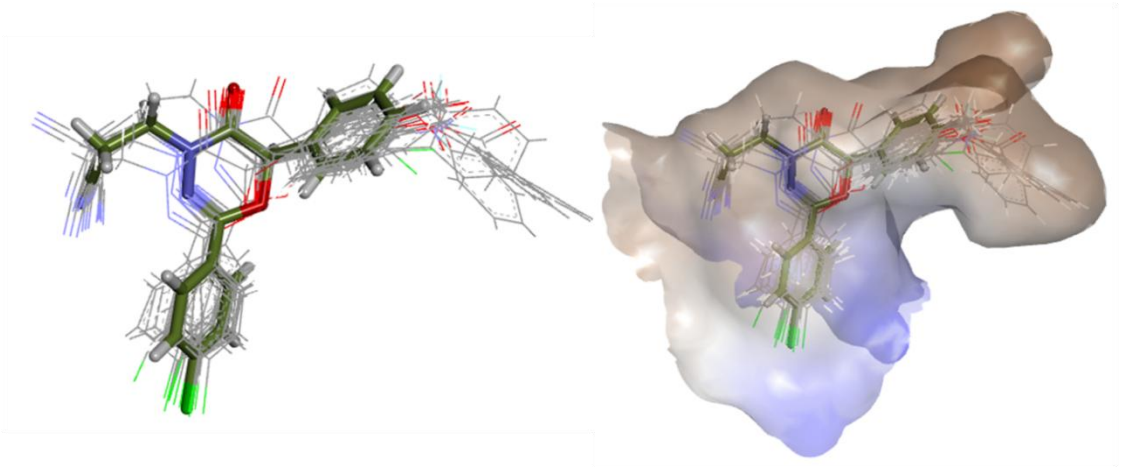


Figure 8. C6위치에 존재하는 다양한 종류의 aromatic ring을 가지는 화합물의 IGF1R에 결합한 modeling 그림

Name	Total_Score	Crash	Polar	Similarity
Reference_PQIP	16.342	-1.1173	2.6707	0.868
22	9.7023	-1.4734	0.6692	0.558
19	9.6753	-0.9073	0.9057	0.538
24	9.0713	-0.8292	0.4012	0.863
25	8.1889	-0.9603	1.142	0.451
23	7.8415	-1.8604	0.7491	0.474
21	7.8199	-1.239	0.8702	0.49
18	7.6884	-1.0912	0.5939	0.445
9	7.212	-0.779	0.9473	0.571
10	7.247	-1.1376	1.3021	0.459
14	6.8965	-0.8864	1.1018	0.449
LL-2003	6.8829	-0.605	1.0381	0.447
11	6.7101	-1.6164	1.2095	0.461
17	6.4159	-0.8191	1.1451	0.553
10	6.2784	-0.4769	1.7118	0.426
13	6.0576	-1.7786	1.2355	0.471
12	7.247	-1.1376	1.3021	0.459
20	5.8991	-0.5134	1.0828	0.45
15	5.8133	-0.7321	0.764	0.445
13	5.7257	-1.4402	0.8549	0.439

Table 4. C6 위치에 아릴치환기가 도입이 된 화합물의 IGF1R에 대한 도킹 후 나타난 도킹스코어

Figure 7에서 새롭게 합성한 화합물들은 IGF1R의 결합자리의 빈 공간을 기존의 LL-2003보다 채워주는 모습을 나타내었다. Table 4의 도킹스코어는 화합물 19, 22, 24에서 IGF1R에 대하여 가장 크게 나타났다. 이는 19, 22, 24가 IGF1R에 대하여 결합력이 가장 크며, LL-2003에 비하여 IGF1R에 대한 결합력이 높음을 확인할 수 있었으며 세 화합물들이 IGF1R의 어떤 아미노산과의 상호작용을 이루는 지를 확인할 수 있었다 (Figure 8). 세 화합물 모두 1,3,4-oxadiazin-5(*6H*)-one의 C6 위치에 존재하는 페닐기 부분이 C lobe의 활성루프 잔기인 Gly1122 그리고 Asp1123 잔기와 N lobe의 문지기(gatekeeper) 잔기인 Met1049와, Met1024 그리고 Val1033 사이에 위치하였고, 마찬가지로 반데르발스 힘에 의한 공통적인 상호작용을 이루고 있다. 또한 Gly1055와의 5Å 거리에서 알킬부분의 nitrile기와의 수소결합의 상호작용을 나타내었다. 게다가 19, 22, 24 모두 PQIP에서 반데르발스 힘의 상호작용을 나타내었지만, LL-2003과 10~12에서는 상호작용을 보이지 않던 결합자리의 활성 아미노산 잔기인 Phe980, Phe1124, Gly1125, Phe1017와 Ala1021은 페닐기에 연결된 작용기들과 반데르발스 힘의 상호작용이 새롭게 나타나고 있다 (Figure 8). 화합물 19, 22, 24에 대하여 IGF1R 포켓 내의 다른 아미노산과의 반데르발스 힘에 의한 결합력이 나타나는 것는 IGF1R에 대하여 세 화합물과 수용체와의 결합력이 증가하였다고 판단할 수 있었다. 그 결과 LL-2003과 10~12보다 19, 22, 24는 항암활성효과에 대해 보다 개선된 효과를 나타낼 수 있을 것으로 기대가 된다.

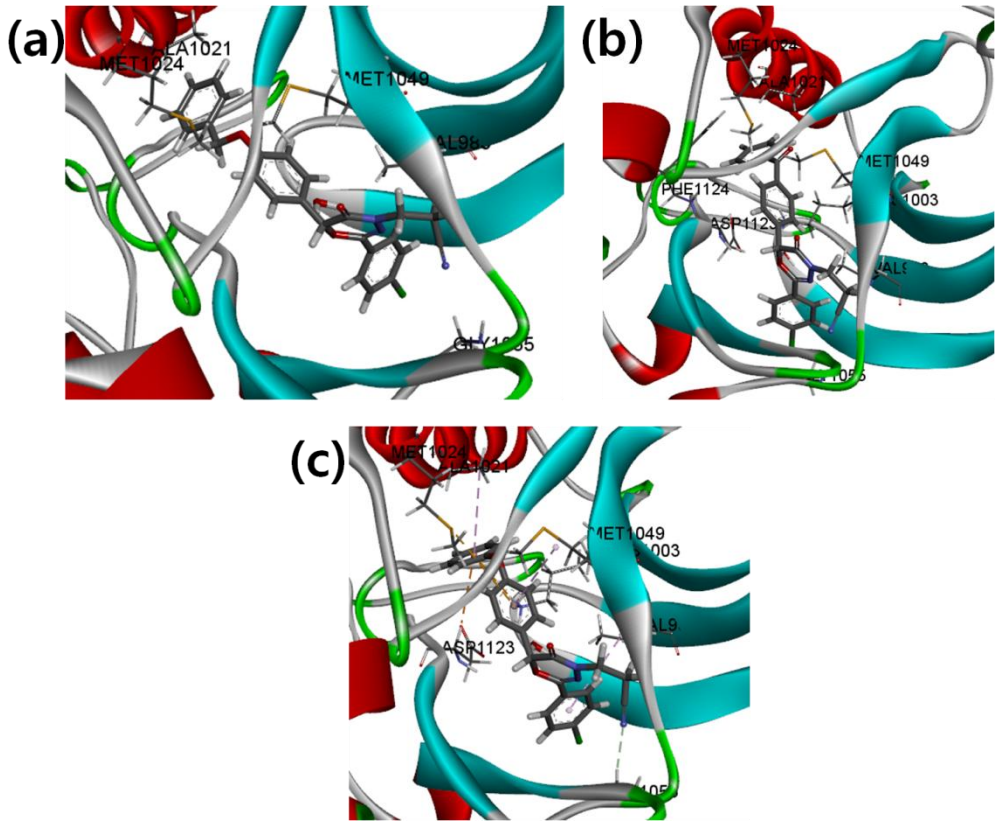


Figure 9. PDB3D94에 도킹한 후, IGF1R과 화합물들과의 상호작용을 나타내는 아미노산 및 크리스탈 구조, 각각에 대해 도킹한 화합물: (a) **19**, (b) **22**, (c) **24**

3. 결론

본 실험실에서는 C6 위치에 다양한 작용기가 도입된 1,3,4-oxadiazin-5(*6H*)-one 유도체를 합성하였다. MTT assay와 분자 모델링을 이용한 도킹을 통하여 IGF1R에 대하여 C6 위치에 알킬 그룹을 가지고 있는 1,3,4-oxadiazin-5(*6H*)-one 화합물의 경우, 아릴 그룹을 가지고 있는 화합물에 비해 활성이 적게 나타났다. 따라서 보다 더 다양한 아릴 그룹을 1,3,4-oxadiazin-5(*6H*)-one의 C6 위치에 도입한 화합물들을 디자인 및 합성하였다. 분자 모델링을 통해 확인한 결과, 작용기의 부피가 증가한 아릴 그룹들은 보다 IGF1R 내의 아미노산들과의 상호작용의 증가로 결합력이 높아진 것을 확인하였고 이는 생리활성에 대한 개선된 효과를 기대할 수 있을 것이다. 하지만 이 연구의 한계점은 존재한다. IGF1R에 대하여 알킬 그룹을 가지고 있는 도킹 스코어 값이 큰

물질의 경우, 아릴 그룹을 가지고 있는 화합물보다 큰 것이 존재할 수는 있었다. 그러나 물질의 항암효과는 알킬 그룹보다는 아릴 그룹을 가지고 있는 화합물에서 나타났다. 그리고 아릴 그룹을 가지는 9~12 중에서 9의 경우는 10~11에 비해 높은 도킹 스코어를 보이나, 10~11에 비해서는 MTT assay에서 낮은 항암활성효과를 나타냈다. 그렇기 때문에 실제로 도킹 결과값이 높은 9, 22, 24가 IGF1R에 대하여 활성이 실제로 있는지에 대해서는 MTT assay를 비롯한 다양한 생물학적 분석을 이용한 활성에 대한 연구가 필요하다. 결과적으로 이 연구는 분자 모델링을 사용하여 다양한 작용기를 도입한 1,3,4-oxadiazin-5(*6H*)-one 유도체를 합성하는 방향을 제시할 수 있고, 항암활성을 나타내는 물질을 합성할 수 있도록 도움을 줄 수 있을 것이다.

4. 참고 문헌

1. Siegel, R. L.; Miller, K. D.; Jemal, A., Cancer statistics, 2016. *CA Cancer J Clin* 2016, *66* (1), 7-30.

2. Jemal, A.; Bray, F.; Center, M. M.; Ferlay, J.; Ward, E.; Forman, D., Global cancer statistics. *CA Cancer J Clin* 2011, *61* (2), 69-90.

3. Geographic Divisions of the American Cancer Society, I., Cancer Facts & Figures 2016. 2016.

4. Krukiewicz, K.; Zak, J. K., Biomaterial-based regional chemotherapy: Local anticancer drug delivery to enhance chemotherapy and minimize its side-effects. *Mater Sci Eng C Mater Biol Appl* 2016, *62*, 927-42.

5. Fuchs-Tarlovsky, V., Role of antioxidants in cancer therapy. *Nutrition* 2013, *29* (1), 15-21.

6. Sun, H.; Wang, J.; Tan, W.; Yang, H.; Li, Y.; Shen, L.; Guo, C., Synthesis and Crystal Structure of a Novel 1,3,4-Oxadiazin-one Compound. *Chinese Journal of Organic Chemistry* 2016, *36* (3), 622.

7. Robert S. Kerbel, P., A Decade of Experience in Developing Preclinical

Models of Advanced- or Early-Stage Spontaneous Metastasis to Study Antiangiogenic Drugs, Metronomic Chemotherapy, and the Tumor Microenvironment. *The Cancer Journal* 2015, *21* (4), 274-283.

8. Iwamoto, T., Clinical Application of Drug Delivery Systems in Cancer Chemotherapy: Review of the Efficacy and Side Effects of Approved Drugs. *Biol. Pharm. Bull* 2013, *36* (5), 715-718.

9. Zhang, J.; Yang, P. L.; Gray, N. S., Targeting cancer with small molecule kinase inhibitors. *Nat Rev Cancer* 2009, *9* (1), 28-39.

10. Baserga, R.; Hongo, A.; Rubini, M.; Prisco, M.; Valentinis, B., The IGF-I receptor in cell growth, transformation and apoptosis. *Biochimica et Biophysica Acta* 1997, *1332*, F105-F126.

11. Wu, J.; Li, W.; Craddock, B. P.; Foreman, K. W.; Mulvihill, M. J.; Ji, Q.-s.; Miller, W. T.; Hubbard, S. R., Small-molecule inhibition and activation-loop trans-phosphorylation of the IGF1 receptor. *The EMBO Journal* 2008, *27*, 1985-1994.

12. Denduluri, S. K.; Idowu, O.; Wang, Z.; Liao, Z.; Yan, Z.; Mohammed, M. K.; Ye, J.; Wei, Q.; Wang, J.; Zhao, L.; Luu, H. H., Insulin-like growth factor (IGF) signaling in tumorigenesis and the development of cancer drug resistance. *Genes Dis* 2015, *2* (1), 13-25.

13. Scagliotti, G. V.; Novello, S., The role of the insulin-like growth factor signaling pathway in non-small cell lung cancer and other solid tumors. *Cancer Treat Rev* 2012, *38* (4), 292-302.

14. Schmitz, S.; Kaminsky-Forrett, M. C.; Henry, S.; Zanetta, S.; Geoffrois, L.; Bompas, E.; Moxhon, A.; Mignion, L.; Guigay, J.; Knoops, L.; Hamoir, M.; Machiels, J. P., Phase II study of figitumumab in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck: clinical activity and molecular response (GORTEC 2008-02). *Ann Oncol* 2012, *23* (8), 2153-61.

15. Bergmann, U.; Funatomi, H.; Yokoyama, M.; Beger, H. G.; Korc, M., Insulin-like Growth Factor I Overexpression in Human Pancreatic Cancer: Evidence for

Autocrine and Paracrine Roles1. *Cancer Research* 1995, *55* (May 15), 2007-2011.

16. Peled, N.; Wynes, M. W.; Ikeda, N.; Ohira, T.; Yoshida, K.; Qian, J.; Ilouze, M.; Brenner, R.; Kato, Y.; Mascaux, C.; Hirsch, F. R., Insulin-like growth factor-1 receptor (IGF-1R) as a biomarker for resistance to the tyrosine kinase inhibitor gefitinib in non-small cell lung cancer. *Cell Oncol (Dordr)* 2013, *36* (4), 277-88.

17. Shin, D. H.; Lee, H. J.; Min, H. Y.; Choi, S. P.; Lee, M. S.; Lee, J. W.; Johnson, F. M.; Mehta, K.; Lippman, S. M.; Glisson, B. S.; Lee, H. Y., Combating resistance to anti-IGFR antibody by targeting the integrin beta3-Src pathway. *J Natl Cancer Inst* 2013, *105* (20), 1558-70.

18. Min, H. Y.; Yun, H. J.; Lee, J. S.; Lee, H. J.; Cho, J.; Jang, H. J.; Park, S. H.; Liu, D.; Oh, S. H.; Lee, J. J.; Wistuba, II; Lee, H. Y., Targeting the insulin-like growth factor receptor and Src signaling network for the treatment of non-small cell lung cancer. *Mol Cancer* 2015, *14*, 113.

19. Ross, D. T.; Perou, C. M., A comparison of gene expression signatures from breast tumors and breast tissue derived cell lines. *Disease Markers* 2001, *17*, 99-109.

20. Xiao, J.; Xu, M.; Hou, T.; Huang, Y.; Yang, C.; Li, J., Dasatinib enhances antitumor activity of paclitaxel in ovarian cancer through Src signaling. *Mol Med Rep* 2015, *12* (3), 3249-56.

21. Sarma, B. K.; Liu, X.; Wu, H.; Gao, Y.; Kodadek, T., Solid phase synthesis of 1,3,4-oxadiazin-5 (6R)-one and 1,3,4-oxadiazol-2-one scaffolds from acyl hydrazides. *Org Biomol Chem* 2015, *13* (1), 59-63.

22. Alam, M. M.; Lee, S. C.; Jung, Y.; Yun, H. J.; Min, H. Y.; Lee, H. J.; Pham, P. C.; Moon, J.; Kwon, D. I.; Lim, B.; Suh, Y. G.; Lee, J.; Lee, H. Y., Novel C6-substituted 1,3,4-oxadiazinones as potential anti-cancer agents. *Oncotarget* 2015, *6* (38), 40598-40610.

23. Lipunova, G. N.; Nosova, E. V.; Charushin, B. N.; Chasovskikh, O. M., Synthesis of fluorinated derivatives of 1,3,4-oxadiazino[6,5,4-i,j]-quinoline. *Khim*

Geterotsikl+ 2001, (10), 1396-1406.

24. Ke, S. Y.; Qian, X. H.; Liu, F. Y.; Wang, N.; Yang, Q.; Li, Z., Novel 4H-1,3,4-oxadiazin-5(6H)-ones with hydrophobic and long alkyl chains: Design, synthesis, and bioactive diversity on inhibition of monoamine oxidase, chitin biosynthesis and tumor cell. *European Journal of Medicinal Chemistry* 2009, *44* (5), 2113-2121.

25. Arikan, N.; Sumengen, D.; Dulger, B., Synthesis and antimicrobial activities of 1,2,4-oxadiazin-5-one, 6-one and 5-thiones. *Turkish Journal of Chemistry* 2008, *32* (2), 147-155.

26. Christl, M.; Bodenschatz, G.; Feineis, E.; Hegmann, J.; Huttner, G.; Mertelmeyer, S.; Schatzlein, K., Cycloadditions of 6H-1,3,4-oxadiazin-6-ones(4,5-diaza-alpha-pyrones) .15. Reactions of 6H-1,3,4-oxadiazin-6-ones with norbornadiene. A new route to 3,6-disubstituted alpha-pyrones. *Liebigs Annalen* 1996, (5), 853-861.

27. Tidwell, T. T.; Sammtleben, F.; Christl, M., Cycloadditions of 6H-1,3,4-oxadiazin-6-ones (4,5-diaza-alpha-pyrones). Part 16. 4-substituted and 4,5-disubstituted methyl 3-phenyl-alpha-pyrone-6-carboxylates from gamma-oxoketenes via alpha,delta-dibromo-delta-lactones. *Journal of the Chemical Society-Perkin Transactions 1* 1998, (13), 2031-2035.

28. Yasui, E.; Wada, M.; Takamura, N., New entry for synthesis of N-acylhydrazones, pyridazinones, and 1,3,4-oxadiazin-6-ones from alpha-amino acid esters. *Chemical & Pharmaceutical Bulletin* 2007, *55* (11), 1652-1654.

29. Hoover, T. R.; Hitchcock, S. R., Oxadiazinones as chiral auxiliaries: diastereoselective aldol addition reactions of N-3-glycolyl-3,4,5,6-tetrahydro-2H-1,3,4-oxadiazin-2-ones. *Tetrahedron-Asymmetry* 2003, *14* (20), 3233-3241.

30. Ke, S. Y.; Wang, K. M.; Zhang, Z. G.; Zhang, Y. N.; Liang, Y.; Shi, L. Q.; Jiang, A. B.; Yang, Z. W., Structural diversity-guided convenient construction of functionalized 4H-1,3,4-oxadiazin-5(6H)-one derivatives. *Research on Chemical Intermediates* 2012, *38* (1), 45-52.

31. Kornet, M. J., Synthesis and anticonvulsant activity of 2-aryl-3,4-dialkyltetrahydro-1,3,4-oxadiazines and 2-aryl-3,4-dialkyltetrahydro-1,3,4-oxadiazin-5-ones. *J Heterocyclic Chem* 1996, *33* (6), 2047-2049.

32. Kudelko, A.; Zielinski, W., Synthesis of novel 1,3,4-oxadiazin-5(6H)-ones and 2-hydroxymethyl-1,3,4-oxadiazoles. *Heterocycles* 2006, *68* (11), 2269-2283.

33. Argyropoulos, N. G.; Corobili, E.; Raptopoulou, E. P., An unusual formation of 1H-s-triazole N-imines from reactions of 1,3,4-oxadiazin-6-ones and nitrilimines. *J Heterocyclic Chem* 1996, *33* (3), 591-597.

34. Geffken, D.; Hansen, F. K.; Khankischpur, M.; Mauz, T.; Meurer, R., Expedient Microwave-Assisted Synthesis of Novel 6-Substituted 5-Alkoxy(benzyloxy)-3,6-dihydro-2H-1,3,4-oxadiazin-2-ones. *Synlett* 2012, (4), 637-639.

Part 2.

C6 위치의 작용기를 가진 1,3,4-oxadiazin-5(*6H*)-one 유도체 합성 및 치환기 효과에 대한 연구

1. 서론

IGF1R(인슐린 유사 성장인자 타입 I 수용체, insulin-like growth factor-type 1 receptor)은 IGF1을 주요 기질로 하는 tyrosine kinase receptor의 일종이다.[10] PDGF, EGF와 IGF 등과 같은 다양한 성장인자들의 복합적인 작용에 의해 IGF1R의 신호경로는 발생한다. IGF1R은 세포성장, 증식, 분화에서 중요한 역할을 하며, 특히 IGF 신호는 근육, 연골, 뼈의 형성과 심근기능과 뇌기능의 유지 등과 같은 생리학적으로도 중요한 역할을 한다.[12-14, 35] 특히 IGF1R의 주요 기능은 세포증식을 촉진하며, 세포자살을 억제하는 역할을 하기 때문에 신생아, 청소년기 성장에 특히 중요하다. 다양한 종양세포에서는 IGF1R의 활성이 증가하는 신호이상을 나타나게 되고 그로 인해 성장인자 신호경로의 활성이 증가하게 된다. 이는 종양세포의 세포증식, 전이 및 침습작용으로 인한 종양이 발생하게 된다.[13, 15-16] 과발현된 IGF1R의 활성을 억제하면 종양에 치료효과가 있을 것이란 연구가 보고되었기 때문에 이는 매우 효과적인 항암치료 전략으로서 타겟이 될 것이다.[14, 17, 36]

1,3,4-oxadiazin-5*(6H)*-one은 단단한 peptoid 형태의 헤테로고리로 solid phase peptoid synthesis에 의해 합성될 수 있다.[21] 1,3,4-oxadiazin-5*(6H)*-one은 monoamine oxidase(MAO)의 억제, 항종양, 항바이러스 및 항세균 활성과 같은 생리활성을 나타낼 수 있다고 보고하였다[23-25]. 특히 항암활성에 대하여 본 실험실에서는 1,3,4-oxadiazin-5*(6H)*-one이 IGF1R과 Src의 억제를 통해 항암활성작용이 나타난 것을 보고하였다. 1,3,4-oxadiazin-5*(6H)*-one에 대하여 C2와 N4위치에 관한 생리활성과 합성에 관한 연구는 많이 보고되었으나[6, 21, 26-33, 37-40], C6위치에 관한 생리활성에 연구는 거의 보고되지 않았다.[34] 본 실험실에서는 1,3,4-oxadiazin-5(*6H*)-one의 N4, C6 위치의 functionalization을 통하여 IGF1R과 Src를 타겟으로 한 실험을 진행을 하였으며,[22] 기존에 본 실험실에서 합성한 LL-2003 **(Figure 1)**은 IGF1R에 대하여 항암활성 효과를 나타났다. 모델링으로 사용한 IGF1R은 ZINC의 PDB3D94를 이용하였고, 화합물들은 IGF1R의 ATP 결합자리에 결합을 한다 **(Figure 1)**. LL-2003의 경우 ATP 결합자리에 결합한 후 구조상 형태를 살펴보면, C6 위치의 페닐기가 위치한 자리에는 비어있는

공간이 존재한다. 페닐기의 파라자리에 다양한 치환기를 도입함으로써 결합자리의 빈 공간을 채워줄 수 있다는 것을 modeling을 통한 docking 과정으로 확인하였다. 이는 결합자리에 대한 결합력이 증가하여 생리활성을 보다 개선할 것으로 예상할 수 있다. 이번 실험에서는 모델링 결과를 이용하여 LL-2003의 C6 위치에 존재하는 페닐기의 파라자리에 치환기를 도입한 화합물들을 합성하였다.

Carbon-Carbon 결합의 세기가 매우 강력하기 때문에 C-C 결합이 끊어지는 것은 쉽게 일어나기가 어렵다. 그럼에도 불구하고 본 연구에서는 C6 위치의 페닐기의 파라자리에 치환기가 도입된 1,3,4-oxadiazin-5(*6H*)-one의 합성 과정에서 C6 자리의 작용기가 떨어진 새로운 헤테로고리 구조를 보유한 1,3,4-oxadiazolone이 동시에 형성이 되었다. 앞서 연구되었던 화합물의 C-C bond의 cleavage에 의해 나타나는 반응으로는 카보닐기 화합물에서 주로 일어났으며, 열역학적 이온화 반응, 안정한 carbanion 또는 carbocation의 생성으로 인한 cleavage, epoxide에 의한 oxidative cleavage, Haller-Bauer, Baeyer-Villiger, haloform 반응 등등의 다양한 방법들로 결합이 끊어지는 것으로 보고되었다.[41-50] 본 실험실에서 진행한 이번 연구에서는 치환기의 종류에 따라 고리화의 반응성 차이가 있는지도 알아보고자 하였다. 또한 기존에 보고되지 않았던 경로로 1,3,4-oxadiazolone 화합물이 생성이 되는 것과 1,3,4-oxadiazin-5(*6H*)-one의 C-C 결합이 끊어지면서 C6 위치의 페닐기가 떨어지는 현상에 대한 원인과 메커니즘에 대하여 규명하고자 하였다.

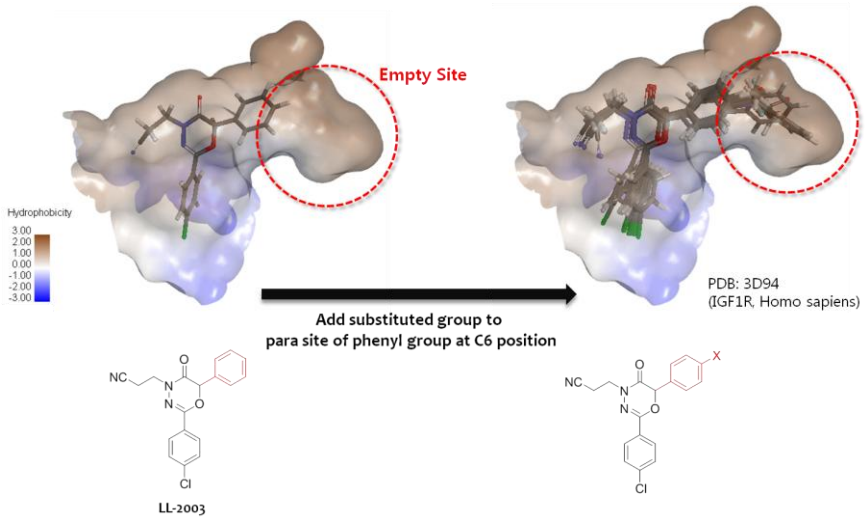
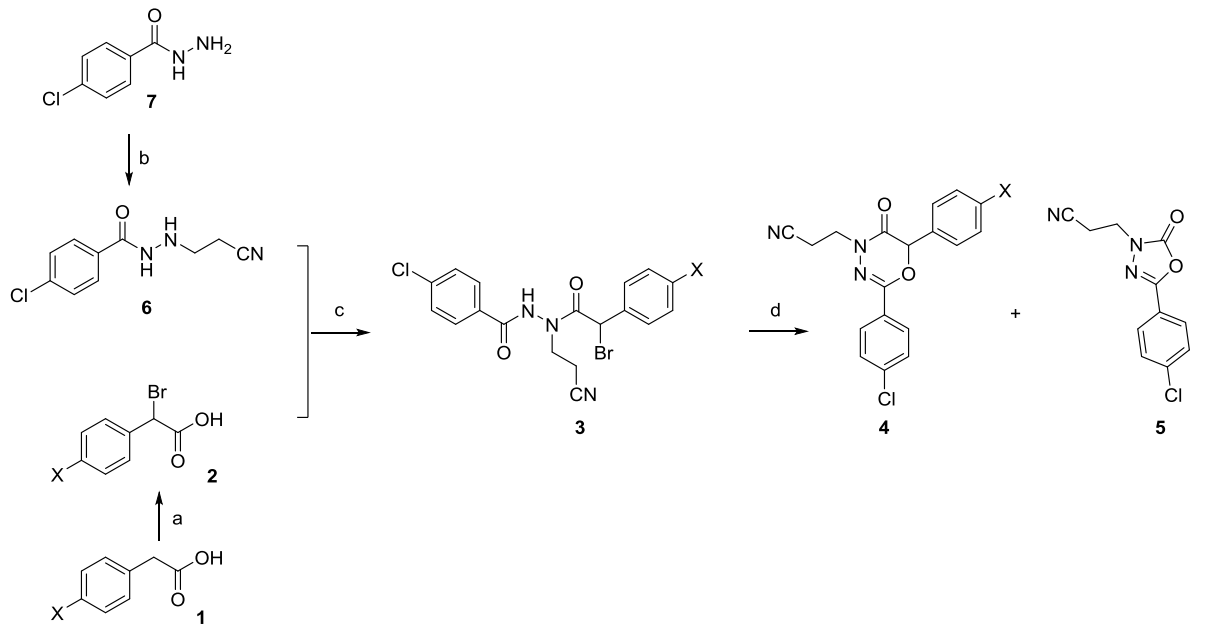


Figure 1. IGF-1R(PDB3D94) Sybyl을 통한 LL-2003과 C6페닐기의 파라위치에 치환기를 도입한 화합물들의 도킹결과

2. 본론

2.1. 합성 전략



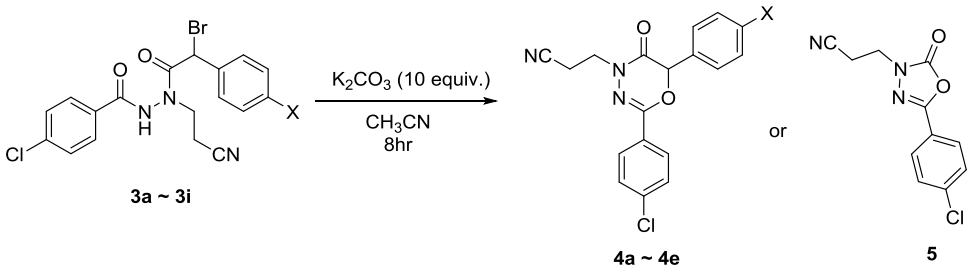
Scheme 1. 1,3,4-oxadiazin-5(6*H*)-one의 전체합성과정.

Reagents and condition: (a) NBS (1 eq.), $(PhCO_2)_2$ (0.05 eq.), Benzene, Reflux, overnight (b) Acrylonitrile (2 eq.), EtOH, Reflux, 72 hr (c) EDC·HCl (1.5 eq.), CH_2Cl_2, RT (Except X=NO_2; Solvent condition is CH_2Cl_2 : EtOAc=1:1), 1 hr (d) K_2CO_3 (10 eq.), CH_3CN, temperature (RT, 50, 75 ℃), 8 hr; (NBS: N-Bromosuccinimide; RT: room temperature)

다음 Scheme 1은 1,3,4-oxadiazin-5(6*H*)-one을 합성하는 방법이다. 1,3,4-oxadiazin-5(6*H*)-one의 C6 자리의 페닐기의 파라위치에 다양한 치환기가 도입되었다. 먼저 파라자리에 치환기가 도입이 된 arylacetic acid **1a~1i**를 NBS를 이용하여 alpha 자리에 브롬을 도입한 결과 **2a~2i**가 합성되었다. 4-chlorobenzohydrazide **7**에 acrylonitrile을 이용한 alkylation을 진행하여 **6**을 합성하였다. 합성된 **2a~2i**와 **6**을 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide을 이용한 EDC coupling을 진행하여 **3a~3i**를

합성하였고, 염기로 K_2CO_3를 이용하여 고리화반응을 진행하여 **4a~4i**를 합성할 수 있었다. 그 결과 1,3,4-oxadiazin-5(6*H*)-one을 모핵으로 가지는 물질이 합성이 되었다. 그런데 (d) 과정을 진행하여 고리화반응을 진행하였을 때 일부 경우, 6각 모핵 1,3,4-oxadiazin-5(6*H*)-one을 가지는 **4a~4i** 뿐 아니라 5각 모핵인 1,3,4-oxadiazolone을 가지는 화합물 **5**가 나타났다. 이는 C6 위치의 페닐기의 파라자리에 존재하는 치환기의 종류에 따라 다르게 나타난 것을 확인하였다.

2.2. 치환기가 electron donating group 일 때의 고리화반응



Scheme 2. 3a~3i의 염기를 이용한 고리화반응

Compound	-X	Hammett Constant (σ)	Temp. [℃]	Yield [a] 4	Yield [a] 5	4 : 5
3a	-OPh	-0.32	RT	85%	ND [b]	4a
			50 ℃	87%	ND [b]	4a
			75 ℃	88%	ND [b]	4a
3b	$-OCH_3$	-0.28	RT	19%	ND [b]	4b
			50 ℃	29%	ND [b]	4b
			75 ℃	42%	ND [b]	4b
3c	-Ph	-0.01	RT	86%	ND [b]	4c
			50 ℃	80%	ND [b]	4c
			75 ℃	84%	ND [b]	4c
3d	-H	0	RT	89%	ND [b]	4d
			50 ℃	92%	ND [b]	4d
			75 ℃	85%	ND [b]	4d
3e	$-OCOCH_3$	0.31	RT	60%	ND [b]	4e
			50 ℃	68%	ND [b]	4e
			75 ℃	63%	ND [b]	4e

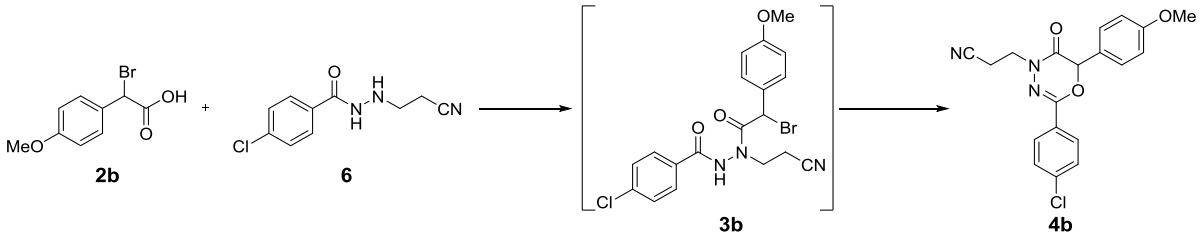
Table 1. C6 위치에 존재하는 페닐기의 파라자리 치환기가 electron donating group일 때의 화합물의 종류.

[a] Yield of the products after isolation

[b] Not detected

다음 Scheme 2와 Table 1은 C6 페닐기의 파라 자리에 Electron donating group을 도입한 3a~3e의 경우를 나타낸 것이다. 이 경우에는 모두 1,3,4-oxadiazinone인 4a~4e의 형성이 우세하였다. 특히 3a와 3b는 염기로 사용되는 K_2CO_3가 존재하지 않을 때에도 고리화가 진행이 되었다. 먼저 치환기로 methoxy (X = MeO)를 도입한 경우,

Scheme 3과 같이 one-pot으로 중간생성물 3b를 빠르게 거쳐 곧바로 4b가 생성이 되는 것을 확인하였다.



Scheme 3. 치환기로 Methoxy를 가지고 있을 때의 EDC coupling 반응. Coupling 반응 과정에서 3b를 거쳐 4b가 곧바로 형성됨.

치환기로 phenoxy (X = PhO)를 도입한 3a는 base를 사용하지 않아도 자발적으로 intermolecular reaction이 진행되어 고리화가 나타났다. 만일 Base를 넣으면 더욱 빠르게 고리화가 1시간 이내 완료가 된다. 3a에서 자발적으로 고리화반응이 진행이 일어나는 반응의 증거는 HPLC의 trace 분석과 UV spectrum의 변화, 그리고 NMR trace의 변화를 관찰하여 확인할 수 있었다.

2.2.1. X = OPh 일 때, 3a 가 4a 로 자발적 고리화반응이 일어나는 증거: HPLC Trace

X = OPh를 치환기로 가지는 3a가 자발적인 고리화반응에 의해 4a로 전환되는지에 대한 실험으로 먼저 역상 크로마토그래피를 이용한 HPLC의 retention time의 차이를 통해 분석을 진행해보았다. 사용한 HPLC 조건은 다음과 같다. 0.1% trifluoroacetic acid (TFA) in CH_3CN과 0.1% TFA in H_2O을 전개용매로 사용하였으며, 용매의 flow rate는 1 mL/min으로 설정하였다. 용매의 gradient를 설정할 수 있었는데, CH_3CN : H_2O은 처음에는 95 : 5로 설정하였다. 샘플을 주입하고 난 15 min 후에는 5 : 95가 되었으며, 20 min 후에는 0 : 100으로 gradient를 변화시켰다. 그 다음으로 25 min까지 95 : 5로 gradient를 조절한 후, 40 min까지 용매의 비율을 95 : 5로 설정하였다.

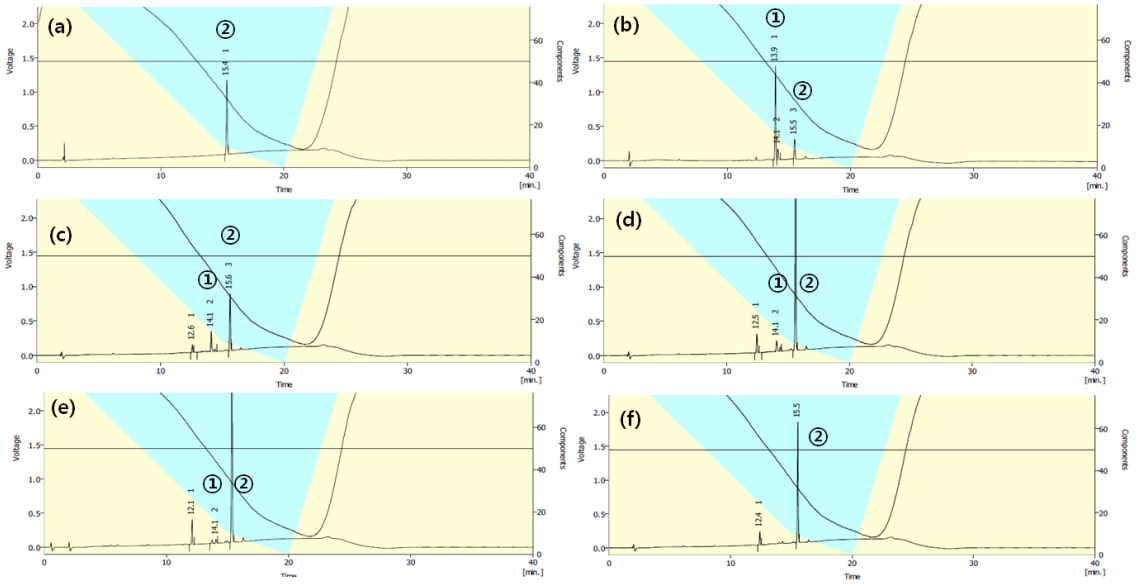


Figure 2. HPLC를 이용한 **3a**(①)의 **4a**(②) 전환을 보이는 증거. (a) **4a**의 HPLC trace; **3a**의 시간에 따라 다른 HPLC trace: (b) 0 hr (c) 6 hr (d) 18 hr (e) 24 hr (f) 30 hr

Figure 2는 HPLC를 이용하여 trace 분석한 결과로 **3a**가 **4a**로 자발적인 고리화반응을 통한 전환이 일어나는지를 확인한 것이다. **Figure 2**에서 각각 **3a**의 retention time은 14.0 min (①)이며, **4a**의 retention time은 15.5 min (②)이다. 2a의 경우, HPLC상에서 측정 시 이미 **3a** : **4a**가 4.5 : 1 비율만큼 **4a**가 형성이 되었다. 시간이 흐르면서 **3a** (①)의 peak의 크기는 점점 감소하며, **4a** (②)의 peak의 크기는 점점 증가한다. 2f는 30 hr이 흐르고 난 후의 HPLC trace의 결과로, 이때 **3a**는 보이지 않고, **4a**만 나타난 것이 확인이 되었다. 이는 **3a**가 자발적으로 고리화반응을 통한 **4a**로의 전환이 이루어졌다는 결과이다.

2.2.2. X = OPh일 때, 3a가 4a로 자발적 고리화 반응이 일어나는 증거: UV-Vis spectrum

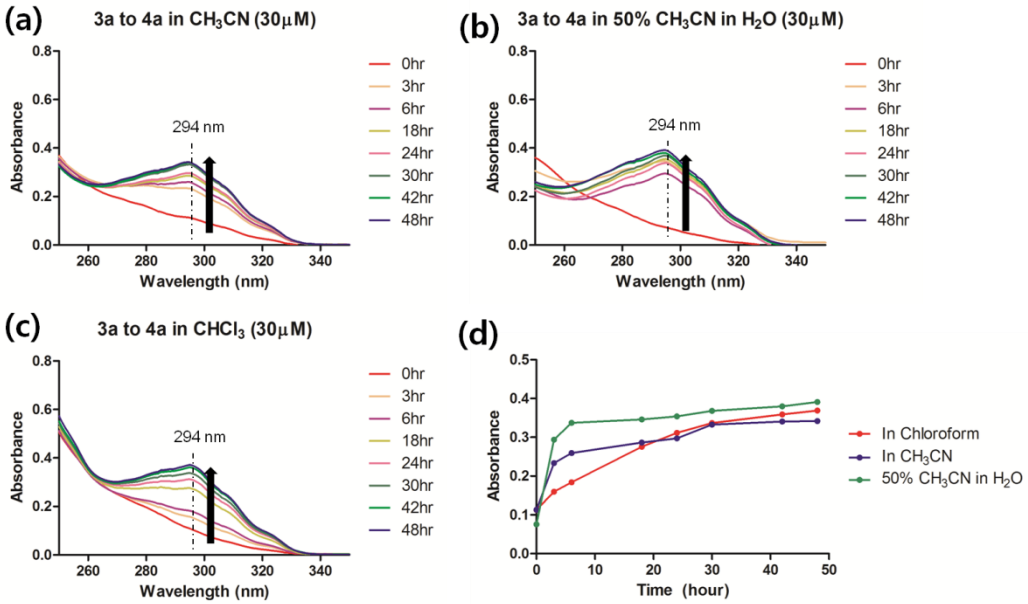


Figure 3. UV를 이용한 **3a** (①)의 **4a** (②) 전환을 보이는 증거. 같은 농도의 3a (30μM) 서로 다른 용매조건에서 자발적 반응이 일어나는지 확인 (a) CH_3CN (b) 50% CH_3CN in H_2O (c) $CHCl_3$ (d) 294nm에서 각 용매 조건에서 증가하는 UV 흡광도의 변화

Figure 3은 294nm에서의 UV흡광도를 이용한 흡수경향성의 차이를 통해 **3a**가 **4a**로 전환이 되는지를 확인하는 실험이다. UV에서 **4a**는 294 nm의 파장에서 최대흡수파장을 나타내는 반면, **3a**는 294 nm에서 파장을 거의 흡수하지 않는다. 즉 고리화반응이 일어났을 때, 최대흡수파장인 294nm에서의 흡광도가 증가한다. 따라서 파장의 흡수하는 정도의 차이를 이용하여, **3a**와 **4a**의 형성되는 정도를 비교할 수 있었다. 최대흡수파장에서의 흡광도가 0.4~0.6의 값을 되도록 30 μM 농도의 **3a**를 사용하였다. Chloroform 용액, acetonitrile 용액, 50% acetonitrile 수용액에서 모두 시간의 흐름에 따라 최대흡수파장 294 nm에서의 흡광도의 크기는 점점 증가하다가, 약 40 hr 이후로는 흡광이 거의 증가하지 않는 결과를 보였다. 3d 그래프를 보았을 때, 294nm의 최대흡수파장에서는 18 hr까지 흡광도가 급격히 증가하며, 이후에는 서서히 흡광도의 증가가 점점 줄어들고, 40 hr 이후로는 거의 증가하지 않는다. 이는 **3a**가 자발적인 고리화반응을 통한 **4a**로의 전환이 이루어질 수 있다는 결과를 나타낸다.

2.2.3. X = OPh일 때, 3a가 4a로 자발적 고리화 반응이 일어나는 증거: NMR trace

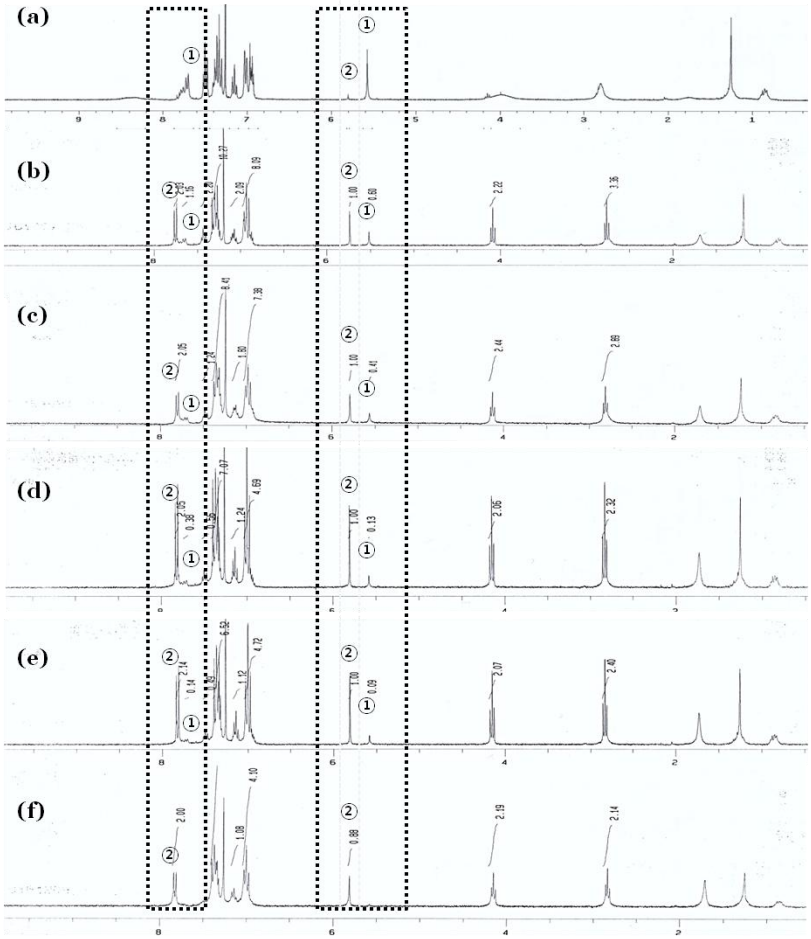


Figure 4. ^{1}H $CDCl_3$ 300MHz에서 측정한 NMR를 이용한 **3a**(①)의 **4a**(②) 전환을 보이는 증거. 5.60 ppm은 **3a**의 peak이고 5.80 ppm은 **4a**의 peak. 시간의 흐름에 따라 **3a**가 **4a**로 전환이 된 것이 확인. 시간 (a) 0 hr (b) 18 hr (c) 24 hr (d) 42hr (e) 48 hr (f) 66hr

분리정제된 **3a**를 $CDCl_3$을 용매로 이용하여 용해시킨 후, 샘플을 시간에 따라 변화하는 ^{1}H-NMR spectrum의 양상을 분석하였다 **(Figure 4).** 각각 화합물 **3a**와 **4a**의 주요 NMR 피크는 C6 위치에 해당하는 proton 자리의 peak로 **Figure 4**의 5.60ppm과 5.80ppm은 ①, ②표시로 각각의 NMR spectrum 위치를 가리킨다. 0 hr에는 (a)에서는 **3a**의 peak에 해당하는 5.60ppm이 거의 주를 이루었다 (**3a** : **4a** = 14.3 : 1). 그러나, 시간이 흐를수록 5.80ppm에 해당하는 **4a**의 peak가 점점 증가하게 되고, 66 hr이 흐른 후에는 **4a**의 5.80ppm에서만 peak가 나타난 것을 확인하였다. 이는 **3a**가 자발적으로 고리화반응을

통한 4a로의 전환이 이루어질 수 있다는 결과이다.

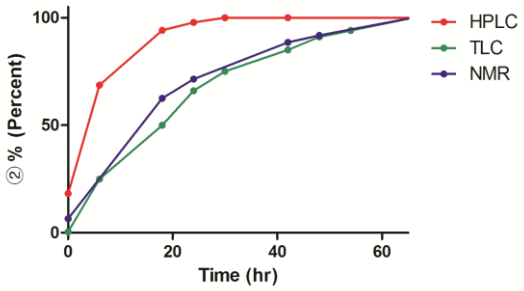


Figure 5. HPLC와 TLC, NMR trace을 통해 3a가 4a로 전환되는 비율

Figure 5 는 UV-Vis spectrum 을 비롯하여, TLC, HPLC 그리고 ^{1}H-NMR ($CDCl_3$, 300 MHz)에서 측정한 **3a** 의 시간의 흐름에 따른 자발적 반응으로 인한 **4a** 로의 전환된 비율을 나타낸 그래프이다. TLC 분석을 통해 자발적 고리화반응이 일어나는 것에 관한 그래프는, TLC 에서의 시간에 따른 **3a** 와 **4a** 의 spot 의 상대적인 진하기를 비교하여 확인할 수 있었다. 결과적으로 모든 경우에서 염기로 사용되는 K_2CO_3 가 존재하지 않았을 때에도 시간이 흐를수록 **3a** 는 자발적으로 고리화반응이 진행되어 **4a** 로 전환되었다고 볼 수 있었다.

2.3. 치환기가 electron withdrawing group일 때의 고리화 반응

Compound	-X	Hammett Constant (σ)	Temp. [℃]	Yield [a] 4	Yield [a] 5	4 : 5 [b]
3f	-COPh	0.46	RT	75%	ND [c]	**4f**
			50 ℃	41%	16%	2.3 : 1 (**4f** : **5**)
			75 ℃	ND	42%	**5**
3g	$-COCH_3$	0.47	RT	98%	ND [c]	**4g**
			50 ℃	49%	9%	5 : 1 (**4g** : **5**)
			75 ℃	11%	30%	1: 3 (**4g** : **5**)
3h	$-CF_3$	0.53	RT	97%	ND [c]	**4h**
			50 ℃	68%	trace	10.5 : 1 (**4h** : **5**)
			75 ℃	39%	24%	1.3 : 1 (**4h** : **5**)
3i	$-NO_2$	0.78	0 ℃	50%	ND [c]	**4i**
			RT	ND	86 %	**5**

Table 2. C6 페닐기의 파라치환기를 electron withdrawing group을 가지고 있는 화합물의 종류.

[a] Yield of the products after isolation

[b] The product ratio was determined by ^{1}H NMR spectroscopic analysis of the crude reaction mixture

[C] Not detected

Scheme 2와 **Table 2**의 **3f~3i**은 페닐기의 파라자리에 elecetron withdrawing group을 도입한 경우로, electron donating group이 도입되었을 때와 마찬가지로 1,3,4-oxadiazin-5(*6H*)-one, **4f~4i**가 형성이 되었다. 그러나 일부 조건에서 1,3,4,-oxadiazion-5(*6H*)-one, **4f~4i** 뿐 아니라 5각형 heterocycle 화합물인 1,3,4-oxadiazolone, **5**가 형성이 되기 시작했다. 치환기의 종류와 온도의 증가에 따라 **5**의 생성비율이 점점 증가하는 경향을 나타내었다. **4**와 **5**의 생성비율은 crude mixture의 NMR spectra를 분석하여 확인할 수 있었다.

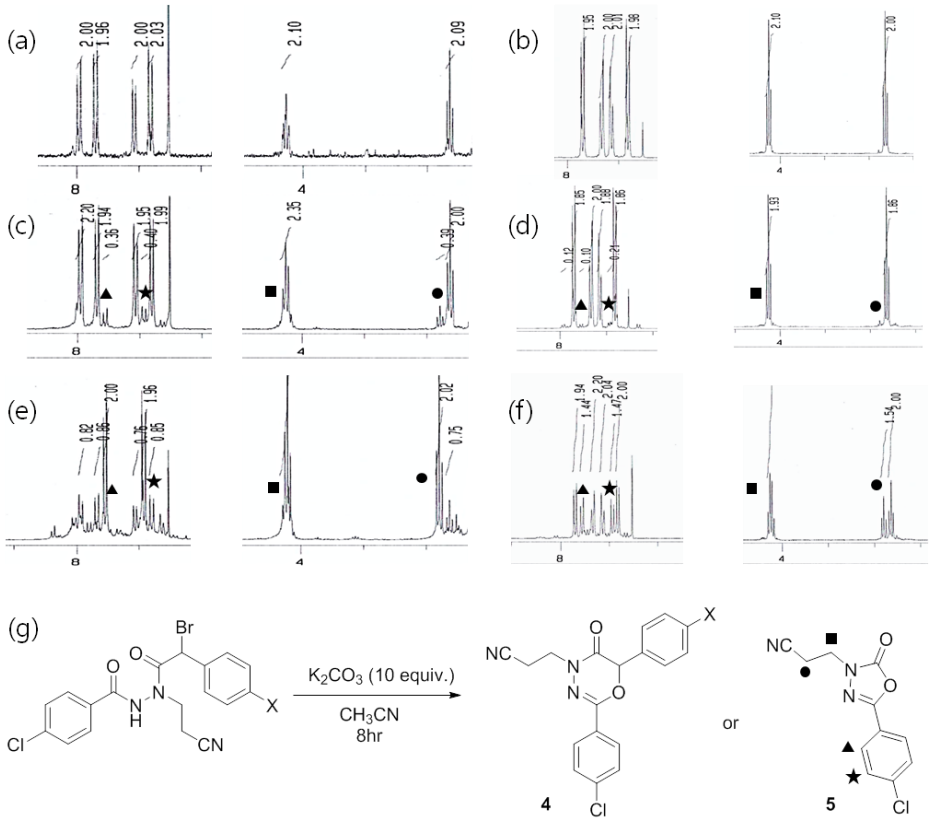
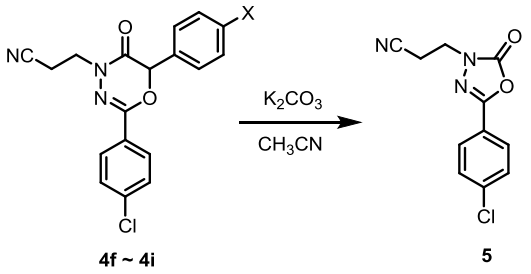


Figure 6. ^{1}H NMR, $CDCl_3$, 300 MHz. (a), (c), (e)는 치환기로 X = $COCH_3$ 였을 때, 반응을 진행; (b), (d), (f)는 치환기로 X = CF_3였을 때 반응을 진행한 것; (a)와 (b)는 온도조건 room temperature, (c)와 (d)는 온도가 50℃, (e)와 (f)는 온도가 75℃ 일 때의 반응, 8hr의 고정된 시간에서 나타난 NMR의 비율; (g) 1,3,4-oxadiazin-5(*6H*)-one과 1,3,4-oxadiazolone의 구조. 1,3,4-oxadiazolone의 aryl과 alkyl 자리의 peak는 NMR spectrum에 각각 도형으로 표시

Base로 K_2CO_3를 이용한 고리화과정을 진행한 후, 확인한 ^{1}H NMR ($CDCl_3$, 300 MHz)을 이용하여 crude mixture의 NMR spectra를 확인해보았다 **(Figure 6)**. 이것은 C6 위치의 페닐기의 파라자리에 존재하는 치환기에 따른 NMR으로, (a), (c), (e)는 $COCH_3$를 가지는 **3g**으로 반응을 진행한 crude mixture이며, (b), (d), (f)는 CF_3를 치환기로 가지는 **3h으**로 반응을 진행한 crude mixture이다. 각각은 다른 온도의 조건으로 반응을 보냈을 때의 결과값으로 충분한 반응이 일어나는 시간이 흘렀을 때, NMR을 비교해보면 RT의 온도조건에서는 6a와 6b 모두 1,3,4-oxadiazolone, **5**가 형성이 되지 않았다. 그러나 75℃라는 보다 높은 온도조건인 6e와 6f에서는 **4g** : **5** = 1 : 3과 **4f** : **5** = 1.3 : 1의 비율로 1,3,4-oxadiazolone, **5**의 형성이 나타났음을 확인할 수 있었다. 그 중간의 온도인 50℃에서는 75℃의 경우보다 1,3,4-oxadiazolone, **5**가

형성된 비율이 적게 나타났다

2.4. 염기를 이용한 Oxadiazinone의 oxadiazolone으로의 전환



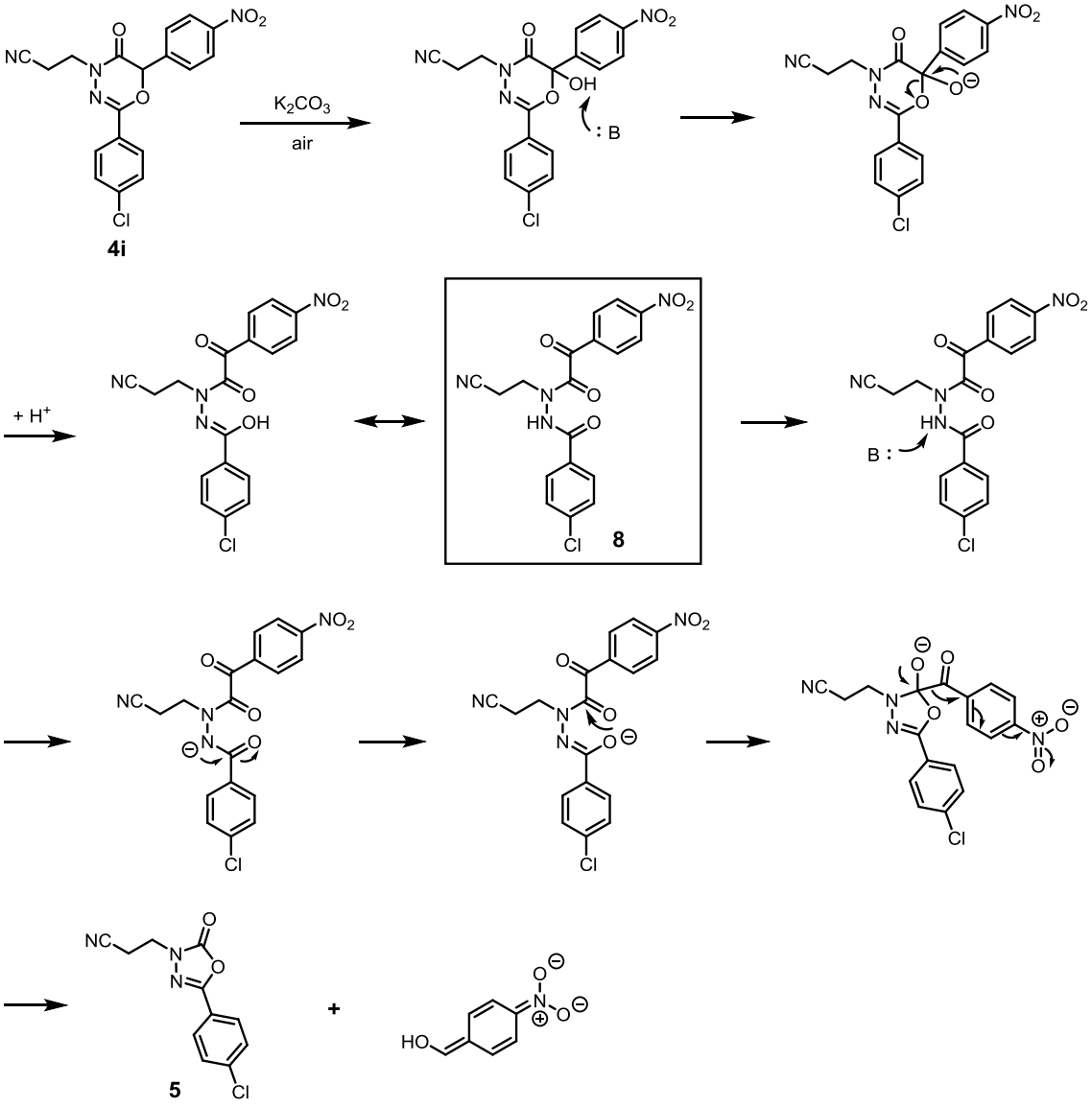
Scheme 4. 치환기로써 electron withdrawing group을 가지고 있을 때 oxadiazinone의 oxadiazolone으로의 전환

Name	-X	K_2CO_3	Ratio	Condition
4f	-COPh	X	Only **4f**	75℃ 8 h
		O	Only **5**	
4g	$-COCH_3$	X	Only **4g**	75℃ 8 h
		O	**4g** : **5** = 1 : 2	
4h	$-CF_3$	X	Only **4h**	75℃ 8 h
		O	**4h** : **5** = 1 : 1.1	
4i	$-NO_2$	X	Only **4i**	RT 8 h
		X	Only **4i**	75℃ 8 h
		O	**5**	RT 8 h

Table 3. 치환기로써 electron withdrawing group을 보유한 oxadiazinone의 oxadiazolone으로의 전환되는 비율

치환기로써 electron withdrawing group을 가지고 있는 1,3,4-oxadiazin-5(*6H*)-one **4f~4i**을 형성하는 고리화 반응에서 1,3,4-oxadiazolone **5**가 함께 생성되는 경우가 나타났다. 그래서 1,3,4-oxadiazin-5(*6H*)-one **4f~4i**가 **5**로 전환되는 것이 아닌지 가정을 하고, 실험을 진행을 하여 Scheme 4과 Table 3과 같은 결과가 나타났다. 먼저

1,3,4-oxadiazin-5(*6H*)-one과 K_2CO_3를 넣지 않은 경우에는 높은 온도에도 **5**로의 전환이 일어나지 않고 안정한 모습을 나타내었다. 그러나 염기인 K_2CO_3를 함께 사용한 경우, **5**가 나타나는 현상이 나타났다. 이는 1,3,4-oxadiazin-5(*6H*)-one이 1,3,4-oxadiazolone으로 전환이 되는 증거가 될 수 있다. 다음 반응이 일어나는 과정에 대한 예상 메커니즘을 추측하여 설계해 보았다 (Scheme 5). 먼저 1,3,4-oxadiazin-5(*6H*)-one의 C6 위치에 K_2CO_3에 의하여 hydroxylation으로 인하여 C6 위치에 hydroxy기의 치환이 진행된다. base가 하이드록시기의 proton을 떼어내면서 헤테로고리가 열리게 된다. 그 결과, diketone을 가지는 hydrazide 구조의 화합물 **8**이 형성이 되는데, 이 화합물은 분리정제를 하여 확인을 할 수 있었다. 다시 염기가 hydrazide의 -NH의 proton을 떼어내는 작용을 하면서, enolate 형태로 전환이 이루어진다. Enolate는 diketone으로 intramolecular 반응을 진행하면서 파라치환된 벤질알코올기가 연속적으로 떨어져나가게 되며 그 결과로 1,3,4-oxadiazolone **5**가 형성이 된다. 떨어진 벤질기는 anion 형태로 떨어지게 되는데 이는 anion의 안정성에 비롯되었다고 판단할 수 있었다. electron withdrawing group을 가진 벤질 음이온의 안정성은 공명 효과와 inductive effect에 기인한다 (Figure 7).[51] 음이온의 안정성의 크기에 따라 compound **4**에서 **5**로의 전환이 빠르게 일어난다고 볼 수 있다.



Scheme 5. 1,3,4-oxadiazin-5(*6H*)-one, **4i**의 C6 자리의 페닐기가 떨어져나가면서 1,3,4-oxadiazolone, **5**로 전환이 되는 메커니즘

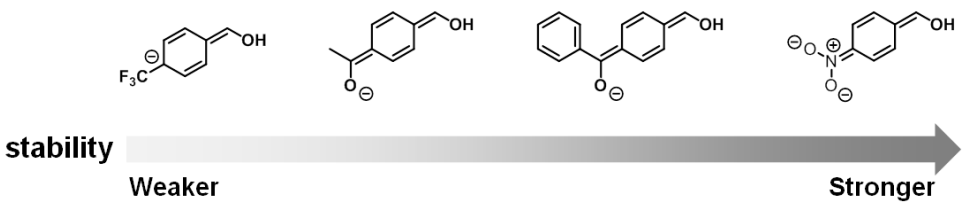


Figure 7. 4가지 electron withdrawing group을 가지고 있는 anion의 안정성의 상대적인 크기

Oxadiazinone을 모핵으로 가지는 **4h**와 oxadiazolone을 모핵으로 가지는 **5**의 X-ray 크리스탈 구조를 확인하였다 **(Figure 8).** 이는 NMR과 Mass spectroscopy를 통한 분석 외에 보다 물질의 구조를 명확히 하는데 사용할 수 있었다.[32, 52]

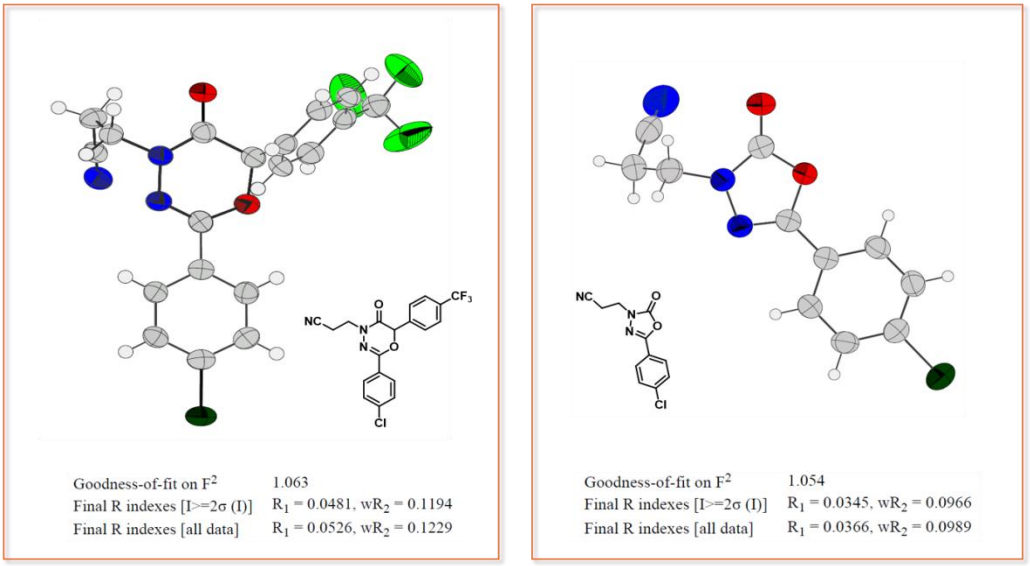


Figure 8. oxadiazinone 4h와 oxadiazolone 5의 X-ray crystal 구조

3. 결론

본 실험실에서는 C6위치의 파라위치에 치환기가 도입된 페닐그룹을 보유하는 1,3,4-oxadiazin-5(*6H*)-one 유도체를 합성하였다. 1,3,4-oxadiazin-5(*6H*)-one 유도체는 C6위치의 페닐기의 파라위치에 도입된 치환기의 종류에 따라 1,3,4-oxadiazolone으로 전환될 수 있었다. Electron donating group를 가진 경우는 1,3,4-oxadiazin-5(*6H*)-one으로 우세하게 진행이 되었다. Electron withdrawing group을 가진 경우에는 oxadiazinone이 먼저 우세하게 형성이 되었으나, 1,3,4-oxadiazin-5(*6H*)-one은 염기가 존재할 때 1,3,4-oxadiazolone으로 전환이 될 수 있었다. 1,3,4-oxadiazin-5(*6H*)-one이 1,3,4-oxadiazolone으로 전환이 되기 위하여 염기인 K_2CO_3과 H_2O은 반드시 필수적인 요소였다. 이 실험에서는 1,3,4-oxadiazin-5(*6H*)-one의 C6 위치에 대한 입체선택성을 고려하지 않아 라세믹 혼합물의 형태로 화합물을 합성하였다. 라세믹 혼합물을 R 형태로 선택적으로 합성해야 하는 부분이 개선해야 할 점이다. 결과적으로 이 연구는

이후에 더 다양한 치환기의 종류를 도입한 1,3,4-oxadiazin-5(*6H*)-one 유도체를 합성하는데 방향을 제시할 수 있을 것이다.

4. 참고문헌

1. Siegel, R. L.; Miller, K. D.; Jemal, A., Cancer statistics, 2016. *CA Cancer J Clin* 2016, *66* (1), 7-30.

2. Jemal, A.; Bray, F.; Center, M. M.; Ferlay, J.; Ward, E.; Forman, D., Global cancer statistics. *CA Cancer J Clin* 2011, *61* (2), 69-90.

3. Geographic Divisions of the American Cancer Society, I., Cancer Facts & Figures 2016. 2016.

4. Krukiewicz, K.; Zak, J. K., Biomaterial-based regional chemotherapy: Local anticancer drug delivery to enhance chemotherapy and minimize its side-effects. *Mater Sci Eng C Mater Biol Appl* 2016, *62*, 927-42.

5. Fuchs-Tarlovsky, V., Role of antioxidants in cancer therapy. *Nutrition* 2013, *29* (1), 15-21.

6. Sun, H.; Wang, J.; Tan, W.; Yang, H.; Li, Y.; Shen, L.; Guo, C., Synthesis and Crystal Structure of a Novel 1,3,4-Oxadiazin-one Compound. *Chinese Journal of Organic Chemistry* 2016, *36* (3), 622.

7. Robert S. Kerbel, P., A Decade of Experience in Developing Preclinical Models of Advanced- or Early-Stage Spontaneous Metastasis to Study Antiangiogenic Drugs, Metronomic Chemotherapy, and the Tumor Microenvironment. *The Cancer Journal* 2015, *21* (4), 274-283.

8. Iwamoto, T., Clinical Application of Drug Delivery Systems in Cancer Chemotherapy: Review of the Efficacy and Side Effects of Approved Drugs. *Biol. Pharm. Bull* 2013, *36* (5), 715-718.

9. Zhang, J.; Yang, P. L.; Gray, N. S., Targeting cancer with small molecule

kinase inhibitors. *Nat Rev Cancer* 2009, *9* (1), 28-39.

10. Baserga, R.; Hongo, A.; Rubini, M.; Prisco, M.; Valentinis, B., The IGF-I receptor in cell growth, transformation and apoptosis. *Biochimica et Biophysica Acta* 1997, *1332*, F105-F126.

11. Wu, J.; Li, W.; Craddock, B. P.; Foreman, K. W.; Mulvihill, M. J.; Ji, Q.-s.; Miller, W. T.; Hubbard, S. R., Small-molecule inhibition and activation-loop trans-phosphorylation of the IGF1 receptor. *The EMBO Journal* 2008, *27*, 1985-1994.

12. Denduluri, S. K.; Idowu, O.; Wang, Z.; Liao, Z.; Yan, Z.; Mohammed, M. K.; Ye, J.; Wei, Q.; Wang, J.; Zhao, L.; Luu, H. H., Insulin-like growth factor (IGF) signaling in tumorigenesis and the development of cancer drug resistance. *Genes Dis* 2015, *2* (1), 13-25.

13. Scagliotti, G. V.; Novello, S., The role of the insulin-like growth factor signaling pathway in non-small cell lung cancer and other solid tumors. *Cancer Treat Rev* 2012, *38* (4), 292-302.

14. Schmitz, S.; Kaminsky-Forrett, M. C.; Henry, S.; Zanetta, S.; Geoffrois, L.; Bompas, E.; Moxhon, A.; Mignion, L.; Guigay, J.; Knoops, L.; Hamoir, M.; Machiels, J. P., Phase II study of figitumumab in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck: clinical activity and molecular response (GORTEC 2008-02). *Ann Oncol* 2012, *23* (8), 2153-61.

15. Bergmann, U.; Funatomi, H.; Yokoyama, M.; Beger, H. G.; Korc, M., Insulin-like Growth Factor I Overexpression in Human Pancreatic Cancer: Evidence for Autocrine and Paracrine Roles1. *Cancer Research* 1995, *55* (May 15), 2007-2011.

16. Peled, N.; Wynes, M. W.; Ikeda, N.; Ohira, T.; Yoshida, K.; Qian, J.; Ilouze, M.; Brenner, R.; Kato, Y.; Mascaux, C.; Hirsch, F. R., Insulin-like growth factor-1 receptor (IGF-1R) as a biomarker for resistance to the tyrosine kinase inhibitor gefitinib in non-small cell lung cancer. *Cell Oncol (Dordr)* 2013, *36* (4), 277-88.

17. Shin, D. H.; Lee, H. J.; Min, H. Y.; Choi, S. P.; Lee, M. S.; Lee, J. W.; Johnson, F. M.; Mehta, K.; Lippman, S. M.; Glisson, B. S.; Lee, H. Y., Combating resistance to

anti-IGFR antibody by targeting the integrin beta3-Src pathway. *J Natl Cancer Inst* 2013, *105* (20), 1558-70.

18. Min, H. Y.; Yun, H. J.; Lee, J. S.; Lee, H. J.; Cho, J.; Jang, H. J.; Park, S. H.; Liu, D.; Oh, S. H.; Lee, J. J.; Wistuba, II; Lee, H. Y., Targeting the insulin-like growth factor receptor and Src signaling network for the treatment of non-small cell lung cancer. *Mol Cancer* 2015, *14*, 113.

19. Ross, D. T.; Perou, C. M., A comparison of gene expression signatures from breast tumors and breast tissue derived cell lines. *Disease Markers* 2001, *17*, 99-109.

20. Xiao, J.; Xu, M.; Hou, T.; Huang, Y.; Yang, C.; Li, J., Dasatinib enhances antitumor activity of paclitaxel in ovarian cancer through Src signaling. *Mol Med Rep* 2015, *12* (3), 3249-56.

21. Sarma, B. K.; Liu, X.; Wu, H.; Gao, Y.; Kodadek, T., Solid phase synthesis of 1,3,4-oxadiazin-5 (6R)-one and 1,3,4-oxadiazol-2-one scaffolds from acyl hydrazides. *Org Biomol Chem* 2015, *13* (1), 59-63.

22. Alam, M. M.; Lee, S. C.; Jung, Y.; Yun, H. J.; Min, H. Y.; Lee, H. J.; Pham, P. C.; Moon, J.; Kwon, D. I.; Lim, B.; Suh, Y. G.; Lee, J.; Lee, H. Y., Novel C6-substituted 1,3,4-oxadiazinones as potential anti-cancer agents. *Oncotarget* 2015, *6* (38), 40598-40610.

23. Lipunova, G. N.; Nosova, E. V.; Charushin, B. N.; Chasovskikh, O. M., Synthesis of fluorinated derivatives of 1,3,4-oxadiazino[6,5,4-i,j]-quinoline. *Khim Geterotsikl+* 2001, (10), 1396-1406.

24. Ke, S. Y.; Qian, X. H.; Liu, F. Y.; Wang, N.; Yang, Q.; Li, Z., Novel 4H-1,3,4-oxadiazin-5(6H)-ones with hydrophobic and long alkyl chains: Design, synthesis, and bioactive diversity on inhibition of monoamine oxidase, chitin biosynthesis and tumor cell. *European Journal of Medicinal Chemistry* 2009, *44* (5), 2113-2121.

25. Arikan, N.; Sumengen, D.; Dulger, B., Synthesis and antimicrobial activities of 1,2,4-oxadiazin-5-one, 6-one and 5-thiones. *Turkish Journal of Chemistry* 2008, *32*

(2), 147-155.

26. Christl, M.; Bodenschatz, G.; Feineis, E.; Hegmann, J.; Huttner, G.; Mertelmeyer, S.; Schatzlein, K., Cycloadditions of 6H-1,3,4-oxadiazin-6-ones(4,5-diaza-alpha-pyrones) .15. Reactions of 6H-1,3,4-oxadiazin-6-ones with norbornadiene. A new route to 3,6-disubstituted alpha-pyrones. *Liebigs Annalen* **1996,** (5), 853-861.

27. Tidwell, T. T.; Sammtleben, F.; Christl, M., Cycloadditions of 6H-1,3,4-oxadiazin-6-ones (4,5-diaza-alpha-pyrones). Part 16. 4-substituted and 4,5-disubstituted methyl 3-phenyl-alpha-pyrone-6-carboxylates from gamma-oxoketenes via alpha,delta-dibromo-delta-lactones. *Journal of the Chemical Society-Perkin Transactions 1* **1998,** (13), 2031-2035.

28. Yasui, E.; Wada, M.; Takamura, N., New entry for synthesis of N-acylhydrazones, pyridazinones, and 1,3,4-oxadiazin-6-ones from alpha-amino acid esters. *Chemical & Pharmaceutical Bulletin* **2007,** *55* (11), 1652-1654.

29. Hoover, T. R.; Hitchcock, S. R., Oxadiazinones as chiral auxiliaries: diastereoselective aldol addition reactions of N-3-glycolyl-3,4,5,6-tetrahydro-2H-1,3,4-oxadiazin-2-ones. *Tetrahedron-Asymmetry* **2003,** *14* (20), 3233-3241.

30. Ke, S. Y.; Wang, K. M.; Zhang, Z. G.; Zhang, Y. N.; Liang, Y.; Shi, L. Q.; Jiang, A. B.; Yang, Z. W., Structural diversity-guided convenient construction of functionalized 4H-1,3,4-oxadiazin-5(6H)-one derivatives. *Research on Chemical Intermediates* **2012,** *38* (1), 45-52.

31. Kornet, M. J., Synthesis and anticonvulsant activity of 2-aryl-3,4-dialkyltetrahydro-1,3,4-oxadiazines and 2-aryl-3,4-dialkyltetrahydro-1,3,4-oxadiazin-5-ones. *J Heterocyclic Chem* **1996,** *33* (6), 2047-2049.

32. Kudelko, A.; Zielinski, W., Synthesis of novel 1,3,4-oxadiazin-5(6H)-ones and 2-hydroxymethyl-1,3,4-oxadiazoles. *Heterocycles* **2006,** *68* (11), 2269-2283.

33. Argyropoulos, N. G.; Corobili, E.; Raptopoulou, E. P., An unusual formation of 1H-s-triazole N-imines from reactions of 1,3,4-oxadiazin-6-ones and nitrilimines. *J*

Heterocyclic Chem 1996, *33* (3), 591-597.

34. Geffken, D.; Hansen, F. K.; Khankischpur, M.; Mauz, T.; Meurer, R., Expedient Microwave-Assisted Synthesis of Novel 6-Substituted 5-Alkoxy(benzyloxy)-3,6-dihydro-2H-1,3,4-oxadiazin-2-ones. *Synlett* 2012, (4), 637-639.

35. Chitnis, M. M.; Yuen, J. S.; Protheroe, A. S.; Pollak, M.; Macaulay, V. M., The type 1 insulin-like growth factor receptor pathway. *Clin Cancer Res* 2008, *14* (20), 6364-70.

36. Ramalingam, S. S.; Spigel, D. R.; Chen, D.; Steins, M. B.; Engelman, J. A.; Schneider, C. P.; Novello, S.; Eberhardt, W. E.; Crino, L.; Habben, K.; Liu, L.; Janne, P. A.; Brownstein, C. M.; Reck, M., Randomized phase II study of erlotinib in combination with placebo or R1507, a monoclonal antibody to insulin-like growth factor-1 receptor, for advanced-stage non-small-cell lung cancer. *J Clin Oncol* 2011, *29* (34), 4574-80.

37. Chang, Y. G.; Kim, K., A facile synthesis of N-arylcyanoformamidoximes, 4-aryl-3-cyano-1,2,4-oxadiazin-5(6H)-ones, 2-cyanoquinazoline-3-oxides, and 2-cyanoquinazolines via 5-arylimino-4-chloro-5H-1,2,3-dithiazoles. *Heterocycles* 1999, *51* (11), 2653-2666.

38. Casper, D. M.; Hitchcock, S. R., An improved procedure for the asymmetric aldol reaction of the titanium enolate of an N-3-propionyl-3,4,5,6-tetrahydro-2H-1,3,4-oxadiazin-2-one. *Tetrahedron-Asymmetry* 2003, *14* (4), 517-521.

39. Casper, D. M.; Burgeson, J. R.; Esken, J. M.; Ferrence, G. M.; Hitchcock, S. R., Toward the development of a structurally novel class of chiral auxiliaries: Diastereoselective aldol reactions of a (1R,2S)-ephedrine-based 3,4,5,6-tetrahydro-2H-1,3,4-oxadiazin-2-one. *Org Lett* 2002, *4* (21), 3739-3742.

40. Sugimoto, O.; Arakaki, T.; Kamio, H.; Tanji, K., The use of a Mitsunobu reagent for the formation of heterocycles: a simple method for the preparation of 3-alkyl-5-aryl-1,3,4-oxadiazol-2(3H)-ones from carboxylic acids. *Chem Commun*

(Camb) 2014, *50* (55), 7314-7.

41. Binder, C. M.; Dixon, D. D.; Almaraz, E.; Tius, M. A.; Singaram, B., A Simple Procedure for C-C Bond Cleavage of Aromatic and Aliphatic Epoxides with Aqueous Sodium Periodate Under Ambient Conditions. *Tetrahedron Lett* 2008, *49* (17), 2764-2767.

42. Karpov, S. V.; Kaukov, Y. S.; Grigor'ev, A. A.; Nasakin, O. E.; Kaukova, O. V.; Tafeenko, V. A., Synthesis of novel polycyano-containing organic ligands via double carbanion cleavage of 1',3'-dioxo-1',3'-dihydrospiro[cyclopropane-1,2'-indene] derivatives. *Org Biomol Chem* 2016, *14* (15), 3758-64.

43. Gao, Q.; Zhu, Y.; Lian, M.; Liu, M.; Yuan, J.; Yin, G.; Wu, A., Unexpected C-C bond cleavage: a route to 3,6-diarylpyridazines and 6-arylpyridazin-3-ones from 1,3-dicarbonyl compounds and methyl ketones. *J Org Chem* 2012, *77* (21), 9865-70.

44. Feng, P.; Sun, X.; Su, Y.; Li, X.; Zhang, L. H.; Shi, X.; Jiao, N., Ceric ammonium nitrate (CAN) catalyzed modification of ketones via two C-C bond cleavages with the retention of the oxo-group. *Org Lett* 2014, *16* (12), 3388-91.

45. Gao, Q.; Liu, S.; Wu, X.; Zhang, J.; Wu, A., Direct Annulation of Hydrazides to 1,3,4-Oxadiazoles via Oxidative C(CO)-C(Methyl) Bond Cleavage of Methyl Ketones. *Org Lett* 2015, *17* (12), 2960-3.

46. Huang, X.; Li, X.; Zou, M.; Song, S.; Tang, C.; Yuan, Y.; Jiao, N., From ketones to esters by a Cu-catalyzed highly selective C(CO)-C(alkyl) bond cleavage: aerobic oxidation and oxygenation with air. *J Am Chem Soc* 2014, *136* (42), 14858-65.

47. Mehta, G.; Venkateswaran, R. V., Haller-Bauer reaction revisited: Synthetic applications of a versatile C-C bond scission reaction. *Tetrahedron* 2000, *56* (11), 1399-1422.

48. Gerfaud, T.; Wei, H. L.; Neuville, L.; Zhu, J. P., Unexpected C-C Bond Cleavage: Synthesis of 1,2,4-Oxadiazol-5-ones from Amidoximes with Pentafluorophenyl or Trifluoromethyl Anion Acting as Leaving Group. *Org Lett* 2011,

13 (23), 6172-6175.

49. Paquette, L. A.; Gilday, J. P., Cleavage of Carbon-Carbon Bonds with High Stereochemical Control .4. Base-Induced Cleavage of Optically-Active Nonenolizable Benzylic Ketones. *Journal of Organic Chemistry* 1988, *53* (21), 4972-4978.

50. Arnett, E. M.; Molter, K., Thermodynamics and Kinetics of Carbon Carbon Bond Formation and Heterolysis through Reactions of Carbocations with Carbanions in Solution. *J Phys Chem-Us* 1986, *90* (3), 383-389.

51. Nakata, K.; Fujio, M.; Nishimoto, K.; Tsuno, Y., Theoretical study of substituent effects on the gas-phase stabilities of phenoxide anions. *Journal of Physical Organic Chemistry* 2013, *26* (2), 115-123.

52. Hitchcock, S. R.; Nora, G. P.; Casper, D. M.; Squire, M. D.; Maroules, C. D.; Ferrence, G. M.; Szczepura, L. F.; Standard, J. M., X-ray crystallographic and C-13 nuclear magnetic resonance studies of 3,4,5,6-tetrahydro-2H-1,3,4-oxadiazin-2-ones derived from ephedrine and pseudoephedrine. *Tetrahedron* 2001, *57* (49), 9789-9798.

SUPPORTING INFORMATION

Seunggun Park, Bumhee Lim, and Jeeyeon Lee

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 08826, Korea

Experimental Procedures and Characterization Data

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Experimental Section

1. General Methods.

1.1. Solvents and reagents

Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated.

1.2. Chromatography

Reactions were monitored by thin layer chromatography (TLC) carried out on Merck silica plate 60 F254, using UV light (254 nm) and anisaldehyde as the visualizing agent. Flash silica gel chromatography was performed using Zeochem silica gel (ZEOprep 60 particle size 43–63 μm) with hexane, ethyl acetate and dichloromethane as eluents.

1.3. Spectra data

^{1}H NMR spectra were recorded in the solvent indicated on a Varian Gemini 2000 spectrometer at 300 MHz, JEOL, JNM-LA300 spectrometer at 300 MHz, JEOL, a Bruker, AVANCE 500 spectrometer at 500 MHz, JNM-ECA-600 spectrometer at 600 MHz or Bruker Avance III HD spectrometer with a 5-mm CPTCI CryoProbe at 800 MHz. ^{13}C NMR spectra were recorded in the solvents indicated on the previously mentioned spectrometers at 125 Mz, 150 MHz, and 200 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are given in hertz (Hz). The following abbreviations were used to explain NMR peak multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

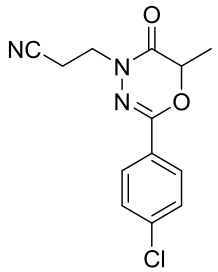
All ESI-MS were undertaken on an Agilent 6130 Single Quadruple LC-MS and high-resolution mass spectra were recorded under fast atom bombardment (FAB) condition on a JEOL, JMS-700 MStation mass spectrometer.

High-performance liquid chromatography (HPLC) spectra were recorded on an YL9100 reversed-phase HPLC.

IR spectra were recorded on a Jasco FT/IR-4200 spectrometer; UV spectra were recorded on a PerkinElmer, Lambda25 UV-Vis spectrometer.

2. [Part 1] Synthesis of 1~25

3-(2-(4-chlorophenyl)-6-methyl-5-oxo -5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propane-nitrile (1)

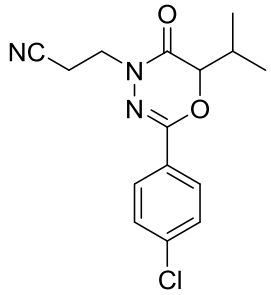


1

A 25 mL round-bottom flask equipped with a stir bar was charged with 2-bromopropanoic acid (44.0 mg, 0.196 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 3) to afford **1** (36.5 mg, 86% yield) as white solid.

R_f = 0.61 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81 (d, 2H, *J* = 8.58 Hz, aryl-CH), 7.39 (d, 2H, *J* = 6.96 Hz, aryl-CH), 4.87 (q, 1H), 4.11-4.05 (m, 2H), 2.80 (t, 2H),1.62 (d, 3H); ESI-MS *m/z* 278.10 $(M+H)^+$

3-(2-(4-chlorophenyl) -6-isopropyl-5-oxo -5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (2)

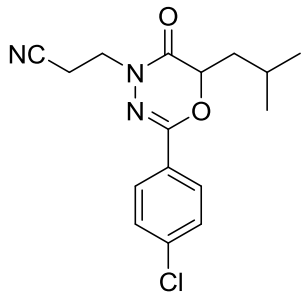


2

A 25 mL round-bottom flask equipped with a stir bar was charged with 2-bromo-3-methylbutyric acid (23.0 mg, 0.125 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 3) to afford **2** (11.4 mg, 30% yield) as white solid.

R_f = 0.43 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, 2H, *J* = 8.58 Hz, aryl-CH) 7.40 (d, 2H, *J* = 6.75 Hz, aryl-CH), 4.64 (d, 1H), 4.21-4.12 (m, 1H), 2.80 (t, 2H), 2.47-2.37 (m, 1H), 1,38 (s, 1H), 1.15 (d, 3H), 1.01 (d, 3H); ESI-MS *m/z* 306.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-6-isobutyl-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (3)

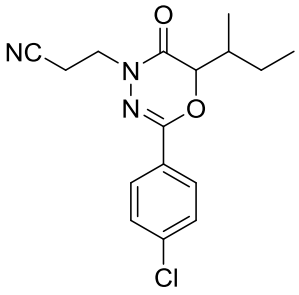


3

A 25 mL round-bottom flask equipped with a stir bar was charged with 2-bromo-4-methylvaleric acid (30.0 mg, 0.154 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 3). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 3) to afford **3** (13.2 mg, 27% yield) as white solid.

R_f = 0.60 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, 2H, *J* = 6.78 Hz, aryl-CH), 7.39 (d, 1H, *J* = 6.78 Hz, aryl-CH), 4.81 (t, 1H), 4.16-3.99 (m, 2H), 2.79 (t, 2H), 1.97-1.90 (m, 1H), 1.83-1.78 (m, 2H), 1.02 (d, 6H); ESI-MS *m/z* 320.10 $(M+H)^+$

3-(6-(*sec*-butyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (4)

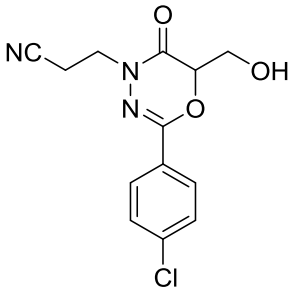


4

A 25 mL round-bottom flask equipped with a stir bar was charged with 2-bromo-3-methylvaleric acid (40.0 mg, 0.205 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 3). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 2) to afford **4** (13.0 mg, 20% yield) as white solid.

R_f = 0.57 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, 2H, *J* = 8.61 Hz, aryl-CH), 7.39 (d, 1H, *J* = 8.61 Hz, aryl-CH), 4.80 (d, 1H), 4.20-4.11 (m, 1H), 4.07-3.98 (m, 1H), 2.80 (t, 2H), 1.66-1.46 (m, 3H), 1.04-0.96 (m, 6H); ESI-MS *m/z* 320.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-6-hydroxymethyl-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (5)

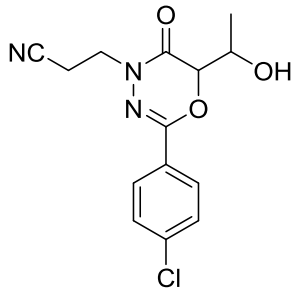


5

A 25 mL round-bottom flask equipped with a stir bar was charged with 2-bromo-3-hydroxypropanoic acid (75.0 mg, 0.444 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 3). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : CH_2Cl_2 : hexane = 1 : 1 : 2) to afford 5 (39.0 mg, 30% yield) as white solid.

R_f = 0.21 (EtOAc : CH_2Cl_2 : hexane = 1 : 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, 2H, *J* = 8.61 Hz, aryl-CH), 7.39 (d, 1H, *J* = 8.61 Hz, aryl-CH), 4.86 (t, 1H), 4.22 (t, 2H), 4.06-3.99 (m, 2H), 2.85-2.79 (m, 2H); ESI-MS *m/z* 294.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-6-(1-hydroxyethyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (6)

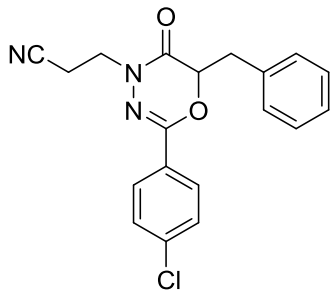


6

A 25 mL round-bottom flask equipped with a stir bar was charged with 2-bromo-3-hydroxybutyric acid (31.0 mg, 0.170 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 2) to afford 6 (7.4 mg, 15% yield) as white solid.

R_f = 0.57 (EtOAc : hexane = 1 : 1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, 2H, *J* = 6.75 Hz, aryl-CH), 7.41 (d, 1H, *J* = 6.78 Hz, aryl-CH), 4.55 (d, 1H), 4.32-4.24 (m, 1H), 4.22-4.15 (m, 1H), 4.04-3.96 (m, 1H), 2.82 (t, 2H), 1.44 (d, 2H); ESI-MS *m/z* 308.10 $(M+H)^+$

3-(6-benzyl-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (7)

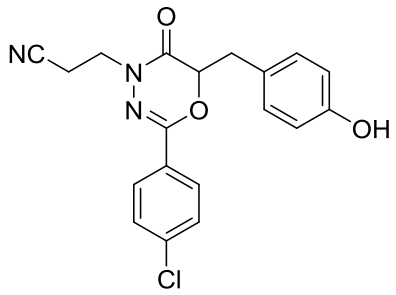


7

A 25 mL round-bottom flask equipped with a stir bar was charged with 2-bromo-3-phenylpropionic acid (30.0 mg, 0.131 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl) carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 3). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 3) to afford **7** (11.8 mg, 28% yield) as white solid.

R_f = 0.52 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, 2H, *J* = 6.78 Hz, aryl-CH), 7.35 (d, 2H, *J* = 8.4 Hz, aryl-CH), 7.28 (m, 3H, aryl-CH), 7.18 (d, 2H, *J* = 5.85 Hz, aryl-CH), 5.06 (t, 1H), 4.11-4.04 (m, 2H), 4.01-3.94 (m, 1H), 3.34-3.13 (m, 2H), 2.68 (t, 2H); ESI-MS *m/z* 354.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-6-(4-hydroxybenzyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (8)

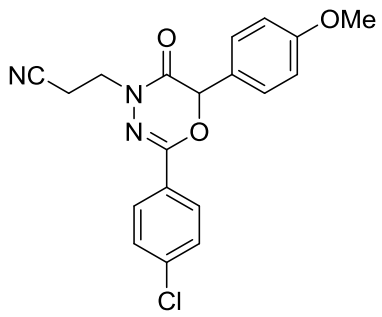


8

A 25 mL round-bottom flask equipped with a stir bar was charged with 2-chloro-3-(4-hydroxyphenyl) propionic acid (80.0 mg, 0.326 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (1.50 eq.) and dichloro-methane (12.5 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 3). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 3) to afford **8** (34.0 mg, 28% yield) as yellow solid.

R_f = 0.29 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H, hydroxyl-OH), 7.69 (d, 2H, *J* = 8.79 Hz, aryl-CH), 7.46 (d, 2H, *J* = 11.19 Hz, aryl-CH), 7.38 (d, 2H, *J* = 8.97 Hz, aryl-CH), 7.10 (d, 2H, *J* = 8.43 Hz, aryl-CH), 5.01 (t, 1H), 4.08-4.00 (m, 2H), 3.32-3.26 (m, 2H), 2.75 (t, 2H); ESI-MS *m/z* 415.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-6-(4-methoxyphenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (9)

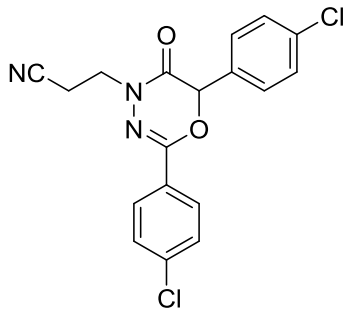


9

A 25 mL round-bottom flask equipped with a stir bar was charged with compound α-bromo-4-methoxyphenylacetic acid (189 mg, 0.772 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 1 hr and was observed by TLC. The organic phases were washed with brine (2 x 50 mL), dried by Na_2SO4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 3) to afford **9** (30 mg, 11% yield) as a bright yellow caramel form.

R_f = 0.62 (EtOAc : hexane = 1 : 1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (t, *J* = 8.6 Hz, 2H, aryl-CH), 7.34 (d, *J* = 8.65 Hz, 2H, aryl-CH), 7.30 (d, *J* = 8.7 Hz, 2H, aryl-CH), 6.88 (d, *J* = 8.75 Hz, 2H, aryl-CH), 5.75 (s, 1H), 4.17-4.10 (m, 2H), 3.77 (s, 3H), 2.81 (t, 2H); ESI-MS *m/z* 370.10 $(M+H)^+$

3-(2,6-bis(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (10)



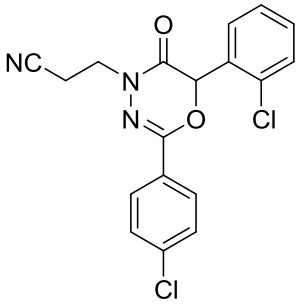
10

A 25 mL round-bottom flask equipped with a stir bar was charged with α-bromo-4-chlorophenylacetic acid (42.5 mg, 0.170 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (1.50 eq.) and dichloromethane (12.5 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by

Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 2) to afford **10** (43.4 mg, 58% yield) as white solid.

R_f = 0.39 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, 2H, *J* = 8.61 Hz, aryl-CH), 7.41-7.35 (m, 6H, aryl-CH), 5.81 (s, 1H), 4.14 (t, 2H), 2.82 (t, 2H); ESI-MS *m/z* 374.10 $(M+H)^+$

3-(6-(2-chlorophenyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (11)



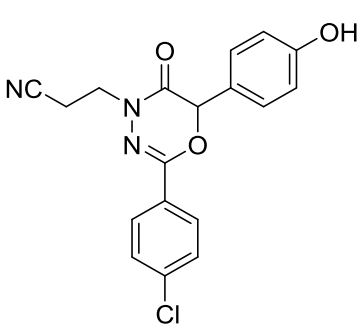
11

A 25 mL round-bottom flask equipped with a stir bar was charged with α-bromo-2-chlorophenylacetic acid (42.5 mg, 0.170 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (1.50 eq.) and dichloromethane (12.5 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 2)

to afford **11** (48.2 mg, 76% yield) as white solid.

R_f = 0.43 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, 2H, *J* = 8.79 Hz, aryl-CH), 7.49 (d, 1H, *J* = 7.32 Hz, aryl-CH), 7.37-7.31 (m, 5H, aryl-CH), 6.18 (s, 1H), 4.25-4.14 (m, 2H), 2.88 (t, 2H); ESI-MS *m/z* 374.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-6-(4-hydroxyphenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (12)

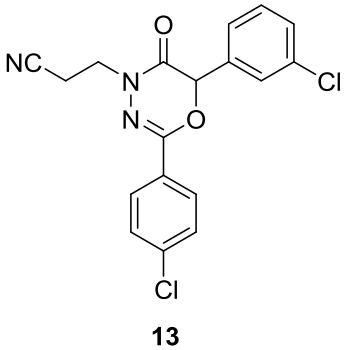


12

A 50 mL round-bottom flask equipped with a stir bar was charged with **21** (102.0 mg, 0.263 mmol, 1.00 eq), 1N NaOH (10 mL) and THF (10mL). The reaction mixture was stirred at room temperature during 30 min and was observed by TLC. The solution was washed with EtOAc (2 x 50 mL), dried by Na_2SO_4 and evaporated to give the purified product **11** (72.3 mg, 77% yield) as white solid.

R_f = 0.38 (EtOAc : hexane = 1 : 1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, 2H, *J* = 8.61 Hz, aryl-CH), 7.37 (d, 2H, *J* = 8.61 Hz, aryl-CH), 7.27 (d, 2H, *J* = 9.72 Hz, aryl-CH), 6.82 (d, 2H, *J* = 8.4 Hz, aryl-CH), 5.76 (s, 1H), 4.97 (s, 1H, Hydroxy-OH), 4.16-4.15 (m, 2H), 2.83 (t, 2H); ESI-MS *m/z* 355.90 $(M+H)^+$

3-(6-(3-chlorophenyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (13)



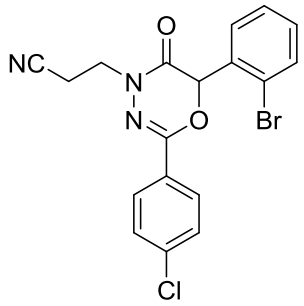
13

A 25 mL round-bottom flask equipped with a stir bar was charged with α-bromo-3-chlorophenylacetic acid (40.0 mg, 0.160 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and

dichloromethane (12.5 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours. and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 2) to afford **11** (38.2 mg, 63% yield) as white solid.

R_f = 0.46 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, 2H, *J* = 8.61 Hz, aryl-CH), 7.42-7.31 (m, 5H, aryl-CH), 5.81 (s, 1H), 4.17-4.11 (m, 2H), 2.82 (t, 2H); ESI-MS *m/z* 373.90 $(M+H)^+$

3-(6-(2-bromophenyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (14)



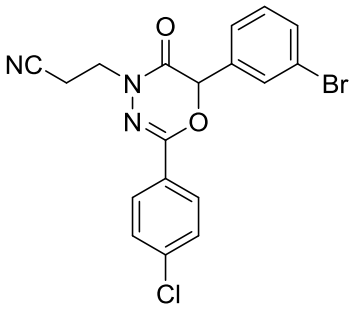
14

A 25 mL round-bottom flask equipped with a stir bar was charged with α-bromo-4-bromophenylacetic acid (30.0 mg, 0.160 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (1.50 eq.) and dichloromethane (12.5 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours. and was observed by TLC then cooled to room temperature. The reaction mixture was

filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 2) to afford **14** (4.0 mg, 10% yield) as white solid.

R_f = 0.45 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, 2H, *J* = 8.4 Hz, aryl-CH), 7.68 (d, 1H, *J* = 7.86 Hz, aryl-CH), 7.37-7.34 (m, 5H, aryl-CH), 6.18 (s, 1H), 4.23-4.17 (m, 2H), 2.89 (t, 2H); ESI-MS *m/z* 419.80 $(M+H)^+$

3-(6-(3-bromophenyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (15)



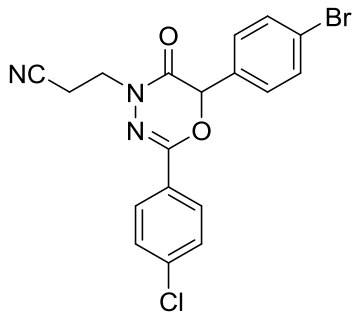
15

A 25 mL round-bottom flask equipped with a stir bar was charged with α-bromo-3-bromophenylacetic acid (39.0 mg, 0.134 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (1.50 eq.) and dichloromethane (12.5 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours. and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 2) to afford **15** (7.3 mg, 13% yield) as white solid.

R_f = 0.44 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, 2H, *J* = 8.76 Hz, aryl-CH), 7.58-7.43 (m, 3H, aryl-CH), 7.40 (d, 2H, *J* = 6.78 Hz, aryl-CH), 7.27 (s, 1H, aryl-CH), 5.80 (s, 1H), 4.19-4.09 (m, 2H), 2.82 (t, 2H); ESI-MS *m/z* 419.80

$(M+H)^+$

3-(6-(4-bromophenyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (16)

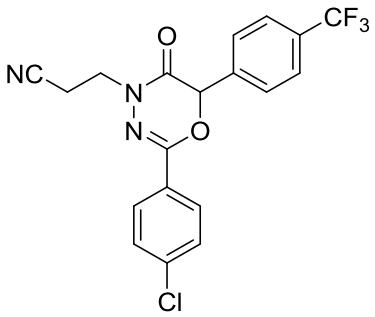


16

A 25 mL round-bottom flask equipped with a stir bar was charged with α-bromo-4-bromophenylacetic acid (20.0 mg, 0.102 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours. and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 2) to afford **16** (7.2 mg, 17% yield) as white solid.

R_f = 0.39 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, 2H, *J* = 8.61 Hz, aryl-CH), 7.53 (d, 2H, *J* = 8.61 Hz, aryl-CH), 7.39 (d, 2H, *J* = 8.79 Hz, aryl-CH), 7.29 (d, 2H, *J* = 8.25 Hz, aryl-CH), 5.80 (s, 1H), 4.12 (m, 2H), 2.82 (t, 2H); ESI-MS *m/z* 419.80 $(M+H)^+$

3-(2-(4-chlorophenyl)-5-oxo-6-(4-trifluoromethylphenyl)-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (17)

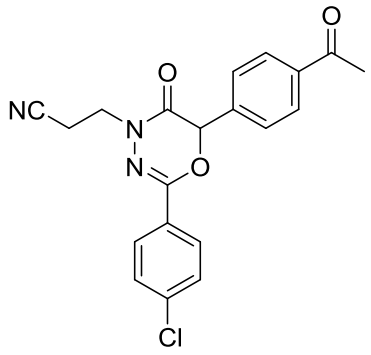


17

A 100 mL round-bottom flask equipped with a stir bar was charged with α-bromo-4-trifluoromethyl-phenylacetic acid (800 mg, 2.827 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloro-methane (30 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 100 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford the purified compound . Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature during 3 hours and was observed by TLC then cooled to room temperature during 8 hours and was observed by TLC. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **17** (185 mg, 44% yield) as white solid.

R_f = 0.52 (EtOAc : hexane = 1 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.6 Hz, 2H, aryl-CH), 7.64 (d, *J* = 8.25 Hz, 2H, aryl-CH), 7.55 (d, *J* = 8.2 Hz, 2H, aryl-CH), 7.39 (d, *J* = 8.65 Hz, 2H ,aryl-CH), 5.89 (s, 1H), 4.11 (t, 2H), 2.80 (t, 2H); ESI-MS *m/z* 408.10 $(M+H)^+$

3-(6-(4-acetylphenyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (18)

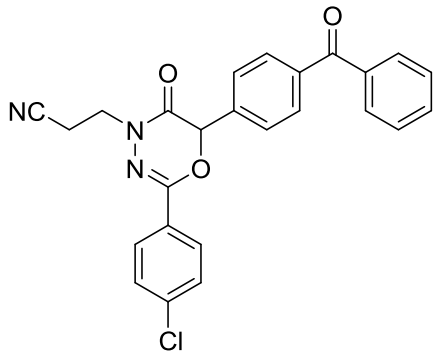


18

A 100 mL round-bottom flask equipped with a stir bar was charged with α-bromo-4-acetylphenylacetic acid (800 mg, 3.115 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloromethane (35 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 100 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford the purified compound . Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature during 3 hours and was observed by TLC then cooled to room temperature during 8 hours and was observed by TLC. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane : CH_2Cl_2 = 1 : 3 : 1) to afford 4g (146.1 mg, 32% yield) as white solid.

R_f = 0.33 (EtOAc : hexane = 1 : 2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 (d, *J* = 6.85 Hz, 2H, aryl-CH), 7.82 (d, *J* = 7.25 Hz, 2H, aryl-CH), 7.52 (d, *J* = 6.85 Hz, 2H, aryl-CH), 7.38 (d, *J* = 8.7 Hz, 2H, aryl-CH), 5.88 (s, 1H), 4.14-4.09 (m, 2H), 2.81-2.78 (m, 2H), 2.56 (s, 3H); ESI-MS *m/z* 382.10 $(M+H)^+$

3-(6-(4-benzoylphenyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (19)

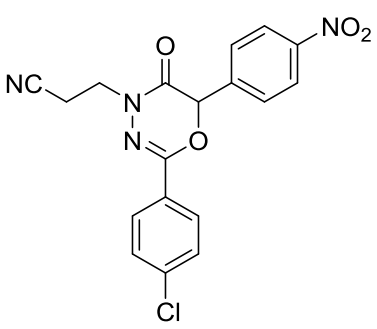


19

A 50 mL round-bottom flask equipped with a stir bar was charged with α-bromo-4-benzoylphenylacetic acid (500 mg, 1.567 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethyl-aminopropyl)carbodiimide HCl (1.50 eq.) and dichloromethane (20 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 75 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford the purified compound . Then, the purified compound was dissolved in acetonitrile (15 mL) with K2CO3 (10.0 eq.). The reaction mixture was stirred at room temperature during 3 hours and was observed by TLC then cooled to room temperature during 8 hours and was observed by TLC. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **19** (80 mg, 32% yield) as white solid.

R_f = 0.42 (EtOAc : hexane = 1 : 2), R_f = 0.43 (EtOAc : hexane : CH_2Cl_2 = 1: 2 : 2); ^1H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.6 Hz, 2H, aryl-CH) , 7.80 (d, *J* = 8.25 Hz, 2H, aryl-CH), 7.75 (d, *J* = 7.45 Hz, 2H, aryl-CH), 7.57 (t, *J* = 7.45 Hz, 1H, aryl-CH), 7.53 (d, *J* = 8.2 Hz, 2H, aryl-CH), 7.45 (t, *J* = 7.7 Hz, 2H, aryl-CH), 7.39 (d, *J* = 8.65 Hz, 2H, aryl-CH, (5.92 (s, 1H), 4.13 (t, 2H), 2.81 (t, 2H); ESI-MS *m/z* 444.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-6-(4-nitrophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (20)

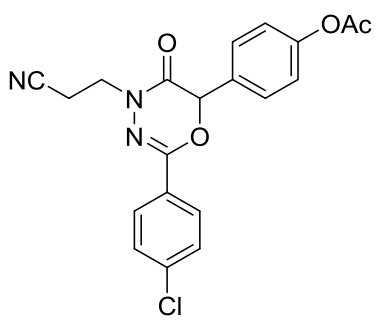


20

A 100 mL round-bottom flask equipped with a stir bar was charged with α-bromo-4-nitrophenylacetic acid (516 mg, 2.307 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.5 eq.) and dichloromethane : EtOAc = 1 : 1 (30 mL). The reaction mixture was stirred at room temperature during 2hr and was observed by TLC. The combined organic phases were washed with brine (2 x 100 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by by silica gel flash chromatography (EtOAc : Hexane = 1: 2) to afford the purified compound . Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 2) to afford **20** (140mg, 40% yield) as white solid.

R_f = 0.39 (EtOAc : hexane = 1 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.7 Hz, 2H, aryl-CH), 7.84 (d, *J* = 8.6 Hz, 2H, aryl-CH), 7.63 (d, *J* = 8.7 Hz, 2H, aryl-CH), 7.40 (d, *J* = 8.6 Hz, 2H, aryl-CH), 5.93 (s, 1H), 4.14-4.07 (m, 2H), 2.82-2.79 (m, 2H); ESI-MS *m/z* 385.09 $(M+H)^+$

4-(2-(4-chlorophenyl)-4-(2-cyanoethyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-6-yl)phenyl acetate (21)

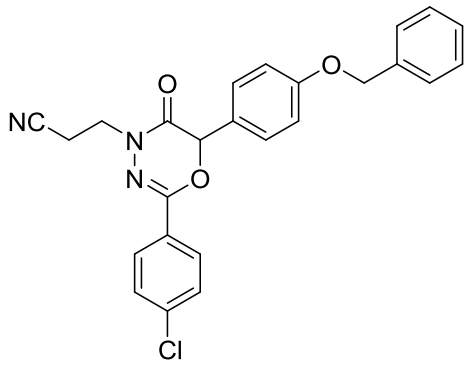


21

A 100 mL round-bottom flask equipped with a stir bar was charged with compound α-bromo-4-acetoxyphenylacetic acid (732 mg, 2.683 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (600 mg, 2.683 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethyl-aminopropyl)carbodiimide HCl (1.50 eq.) and dichloro-methane (30 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 100 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford the purified compound . Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature during 3 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **21** (136.7mg, 53% yield) as white solid.

R_f = 0.32 (EtOAc : hexane = 1 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.7 Hz , 2H, aryl-CH), 7.41 (d, *J* = 8.55 Hz, 2H, aryl-CH), 7.37 (d, *J* = 8.7 Hz, 2H, aryl-CH), 7.10 (d, *J* = 8.6 Hz), 2H, aryl-CH), 5.82 (s, 1H), 4.15-4.05 (m, 2H), 2.78 (t, 2H), 2.26 (s, 3H); ESI-MS *m/z* 398.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-5-oxo-6-(4-benzyloxyphenyl)-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (22)

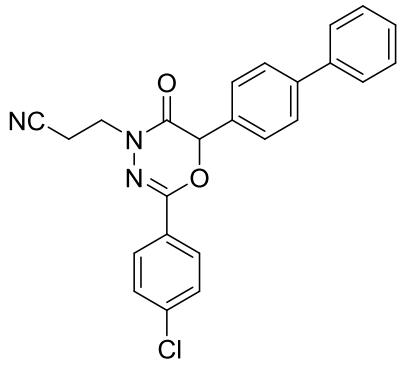


22

A 10 mL round-bottom flask equipped with a stir bar was charged with **12** (15.0 mg, 0.0422 mmol, 1.00 eq), Benzyl bromide (1.5 eq.), K_2CO_3 (3.00 eq.), and acetone (5 mL). The reaction mixture was stirred at reflux during 5 hours and was observed by TLC. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 2) to afford **22** (11.1 mg, 60 %) as white solid.

R_f = 0.33 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, 2H, *J* = 8.61 Hz, aryl-CH), 7.39-7.30 (m, 9H, aryl-CH), 6.97 (d, 2H, *J* = 8.97 Hz, aryl-CH), 5.77 (s, 1H), 5.04 (s, 2H), 4.15 (t, 2H), 2.83 (t, 2H); ESI-MS *m/z* 445.90 $(M+H)^+$

3-(6-([1,1'-biphenyl]-4-yl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (23)



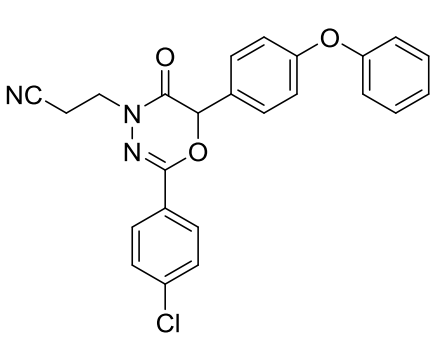
23

A 50 mL round-bottom flask equipped with a stir bar was charged with α-bromo-4-biphenylacetic acid (495 mg, 1.699 mmol, 1.00 eq.), c4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloromethane (20 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 75 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to

afford the purified compound . Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature during 3 hours and was observed by TLC then cooled to room temperature during 8 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford 4c (91 mg, 30% yield) as white solid.

R_f = 0.70 (EtOAc : hexane = 1 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.6 Hz, 2H, aryl-CH), 7.59 (d, *J* = 8.2 Hz, 2H, aryl-CH), 7.53 (d, *J* = 7.35 Hz, 2H, aryl-CH), 7.46 (d, *J* = 8.15 Hz, 2H, aryl-CH), 7.42 (t, *J* = 7.45 Hz, 2H, aryl-CH), 7.36 (d, *J* = 7.45 Hz, 2H, aryl-CH), 7.34 (t, *J* = 7.2 Hz, 1H, aryl-CH), 5.87 (s, 1H), 4.18-4.12 (t, 2H), 2.82 (t, 2H); ESI-MS *m/z* 416.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-5-oxo-6-(4-phenoxyphenyl)-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (24)



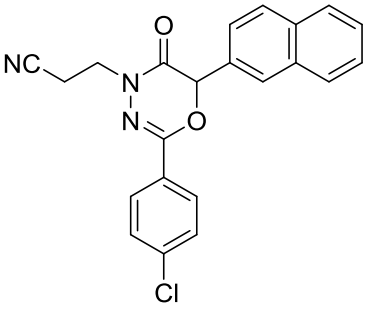
24

A 50 mL round-bottom flask equipped with a stir bar was charged with α-bromo-4-phenoxyphenylacetic acid (307 mg, 1.000 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl) hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethyl-aminopropyl)carbodiimide HCl (1.5 eq.) and di-chloromethane (10 mL). The reaction mixture was stirred at room temperature during 1 hr and was observed by TLC. The organic phases were washed with brine (2 x 50 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford the purified compound . Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The reaction mixture was

stirred at room temperature during 3 hours and was observed by TLC then cooled to room temperature and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **24** (81 mg, ,53% yield) as white solid.

R_f = 0.63 (EtOAc : hexane = 1 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.55 Hz, 2H, aryl-CH), 7.37-7.31 (m, 6H, aryl-CH), 7.12 (t, *J* = 7.35 Hz, 1H, aryl-CH), 6.99 (2H, *J* = 8.25 Hz, 2H, aryl-CH), 6.97 (2H, *J* = 8.7 Hz, 2H, aryl-CH), 5.79 (s, 1H), 4.13 (t, 2H), 2.81 (t, 2H); ESI-MS *m/z* 432.10 $(M+H)^+$

3-(2-(4-chlorophenyl)-6-(naphthalen-2-yl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (25)



25

A 25 mL round-bottom flask equipped with a stir bar was charged with α-bromo-2-naphthylacetic acid (50.0 mg, 0.189 mmol, 1.00 eq.), 4-chlorophenyl-2-(2-cyanoethyl)hydrazine (1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (1.50 eq.) and dichloromethane (12.5 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 25 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 1). Then, the purified compound was dissolved in acetonitrile (15 mL) with K_2CO_3 (10.0 eq.). The mixture was stirred at 50 ºC during 3 hours. and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography chromatography (EtOAc : hexane = 1 : 2) to afford **25** (30.8 mg, 42% yield) as white solid.

R_f = 0.33 (EtOAc : hexane = 1 : 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89-7.81 (m, 6H, aryl-CH), 7.54-7.50 (m, 3H, aryl-CH), 7.38 (d, 2H, *J* = 8.61 Hz, aryl-CH), 6.01 (s, 1H), 4.23-4.15 (m, 2H), 2.85 (t, 2H); ESI-MS *m/z* 389.90 $(M+H)^+$

3. Docking Study Method

Target PDB: 3D94 (IGF1R, Homo sapiens)

PDB's Status: Termini Treatment - N,C-Termini: Charged

Add Hydrogen: H-Bonding

Add Charge: Amber7 FF99, Gasteiger-Marsili

Fix side-chain amides

- Minimization : Method - Powell
- Termination : Gradient, 0.001Kcal/mol*A

Max Iteraction : 10000

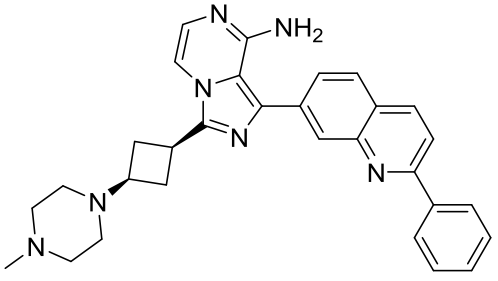
Reference ligand : 3D94 ligand (PQIP)

Modify : Force - Tripos

Charge - Use Current

- Water : H_2O 1354

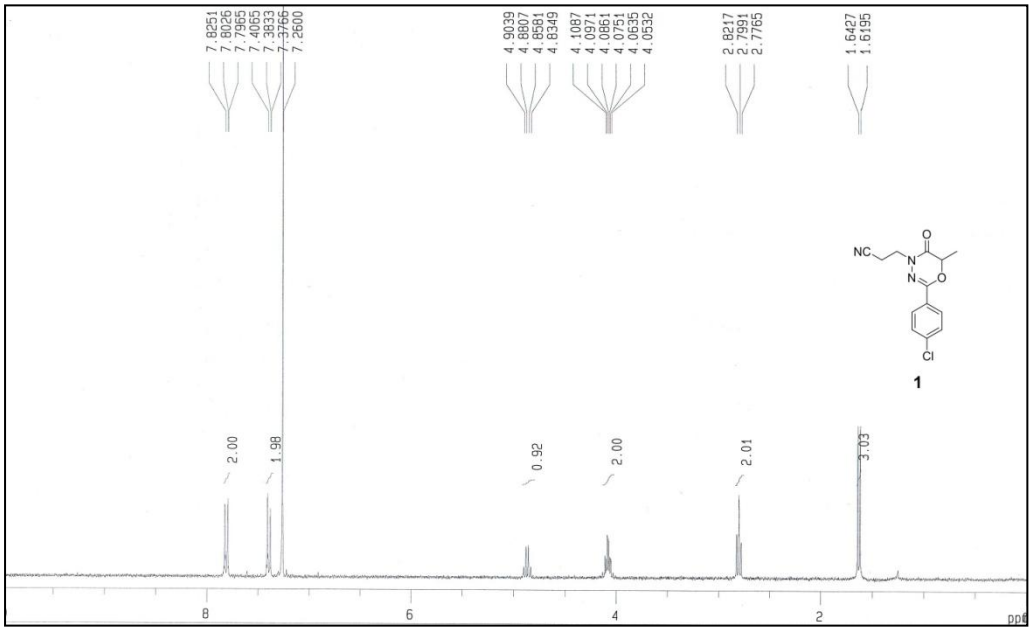
Docking Simulation : Surflex-Dock Geom



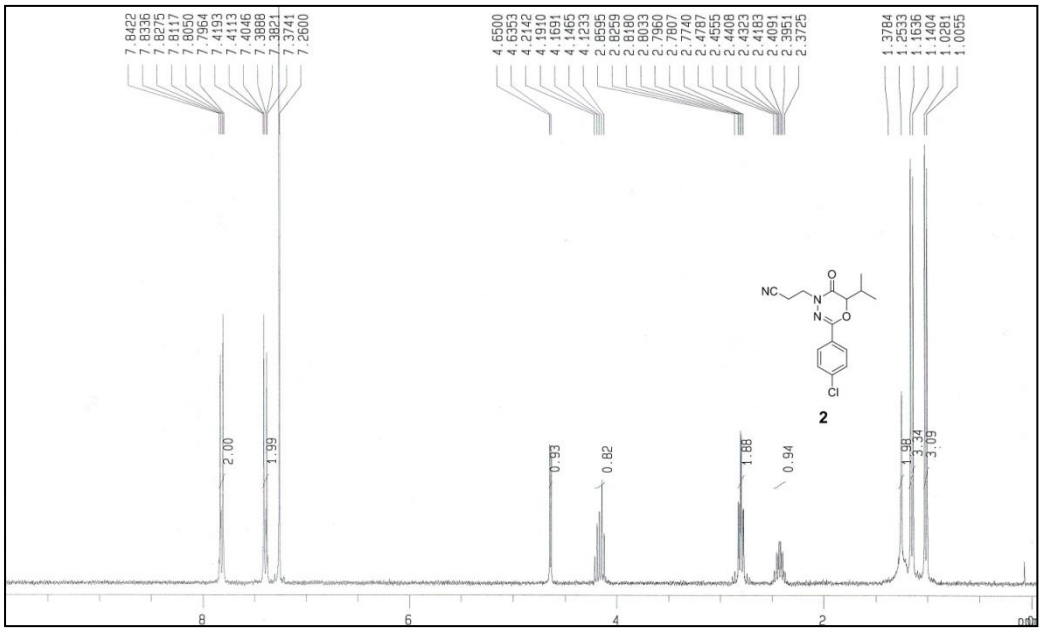
Structure of PQIP: cis-3-[3-(4-methyl-piperazin-l-yl)-cyclobutyl]-1-(2-phenyl-quinolin-7-yl)-imidazo[1,5-a]pyrazin-8-ylamine.

4. [Part 1] NMR Spectra

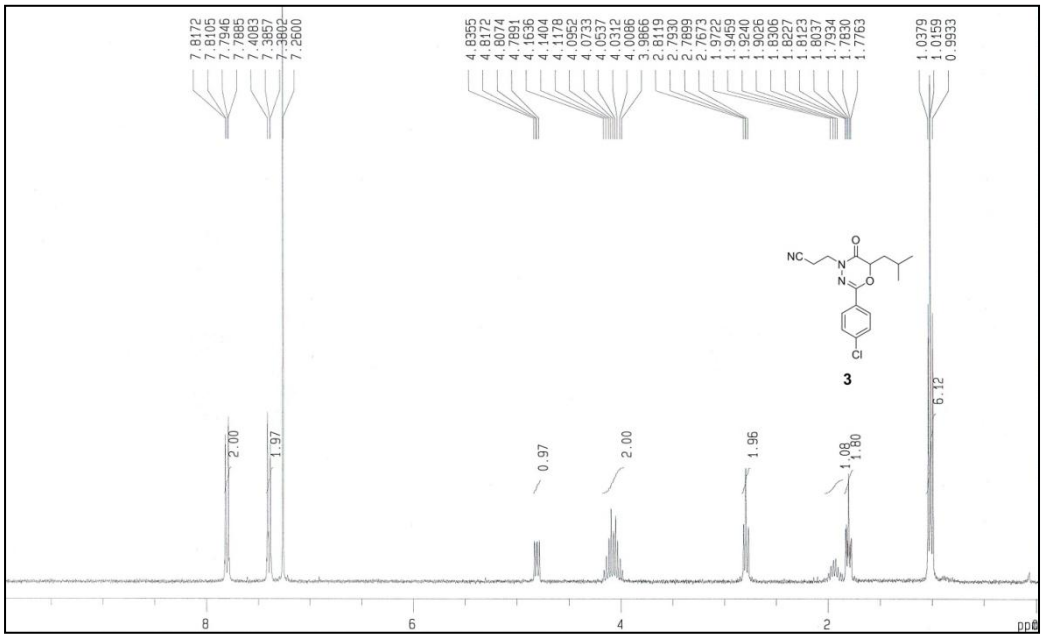
^{1}H NMR of compound **1** (300 MHz, $CDCl_3$)



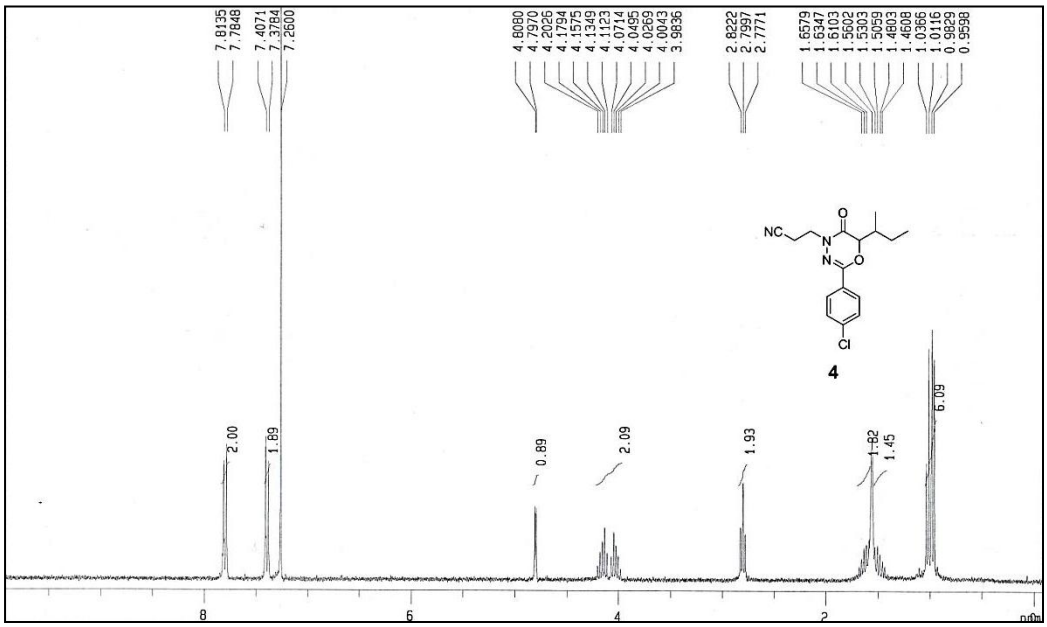
^{1}H NMR of compound **2** (300 MHz, $CDCl_3$)



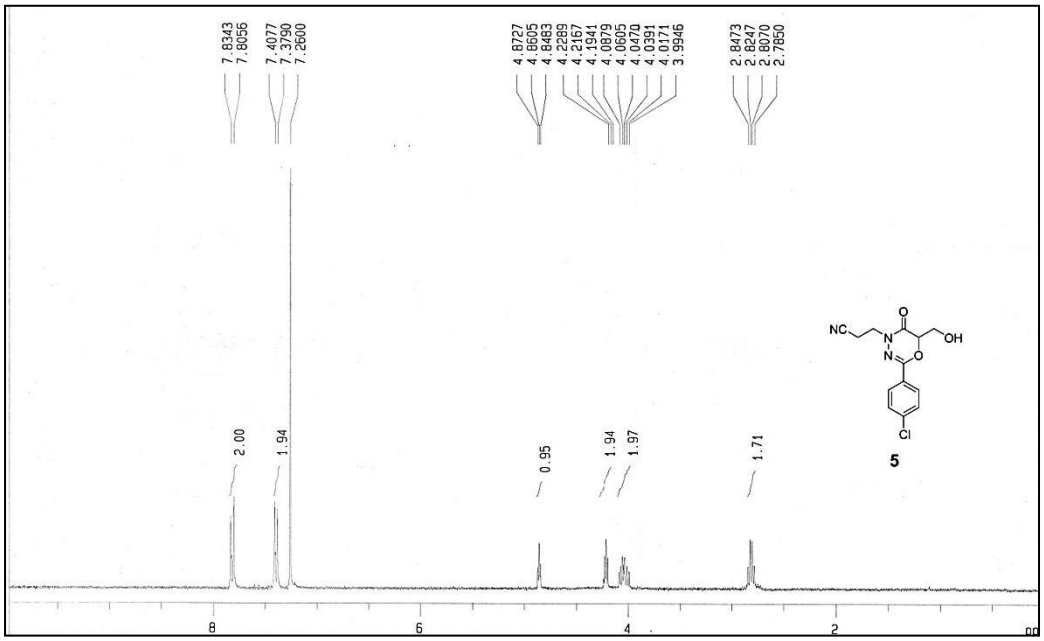
^{1}H NMR of compound **3** (300 MHz, $CDCl_3$)



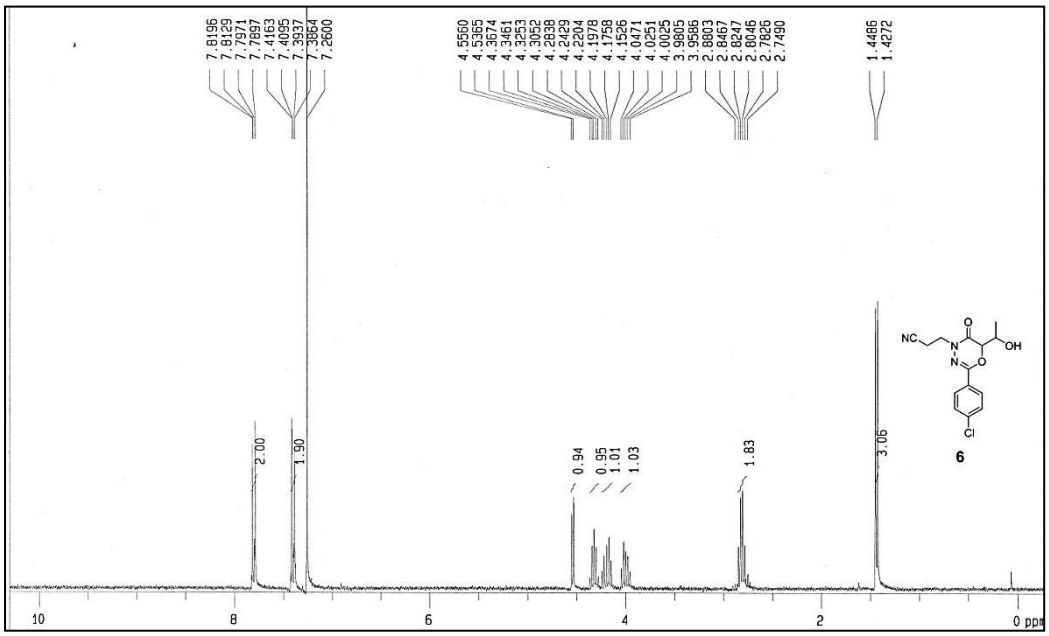
^{1}H NMR of compound **4** (300 MHz, $CDCl_3$)



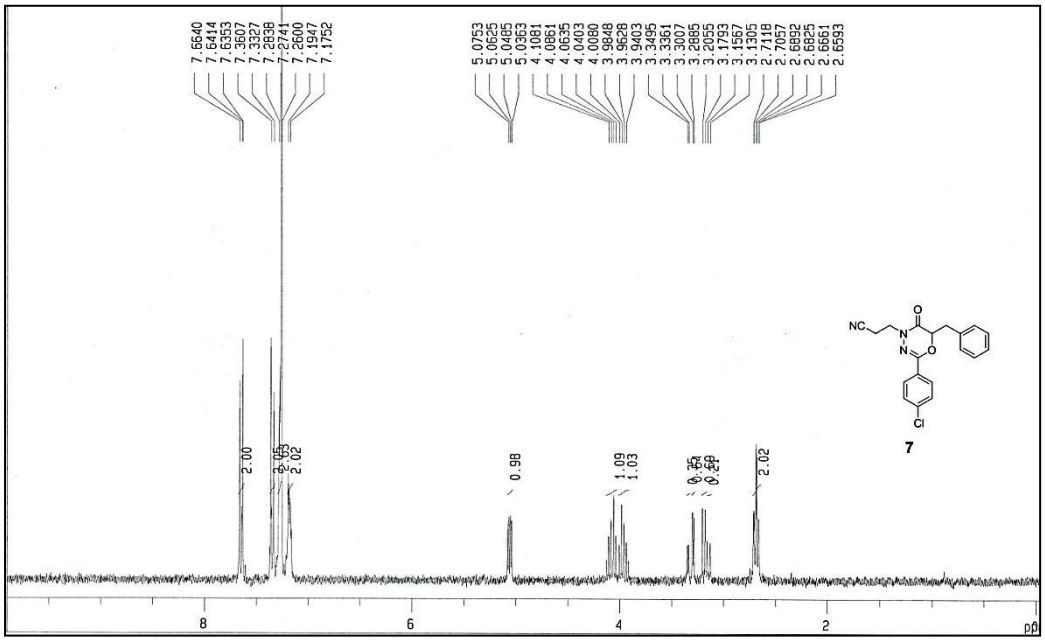
^{1}H NMR of compound **5** (300 MHz, $CDCl_3$)



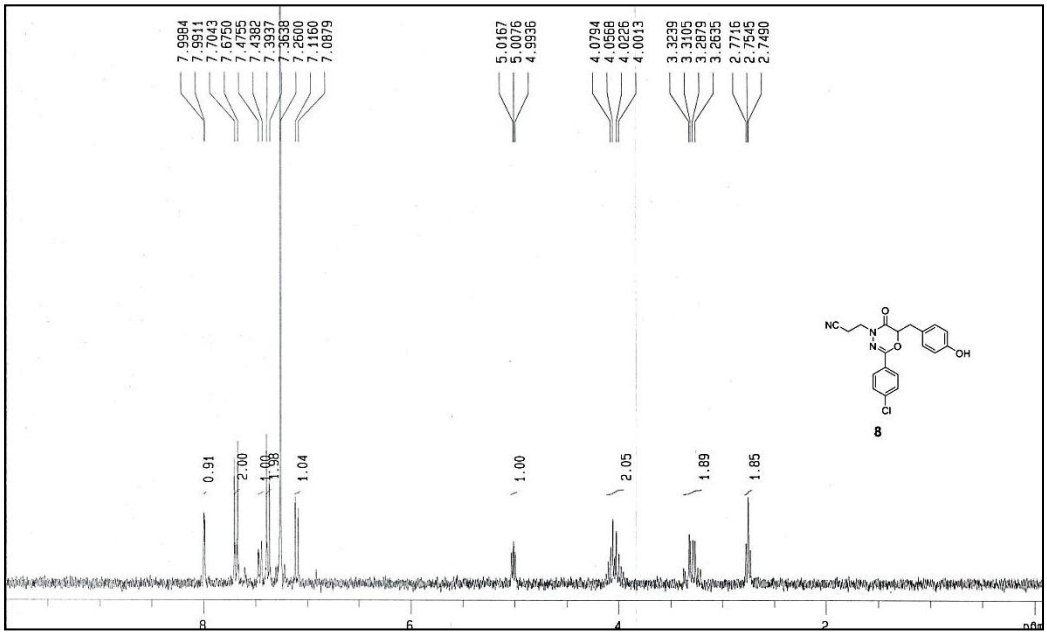
^{1}H NMR of compound **6** (300 MHz, $CDCl_3$)



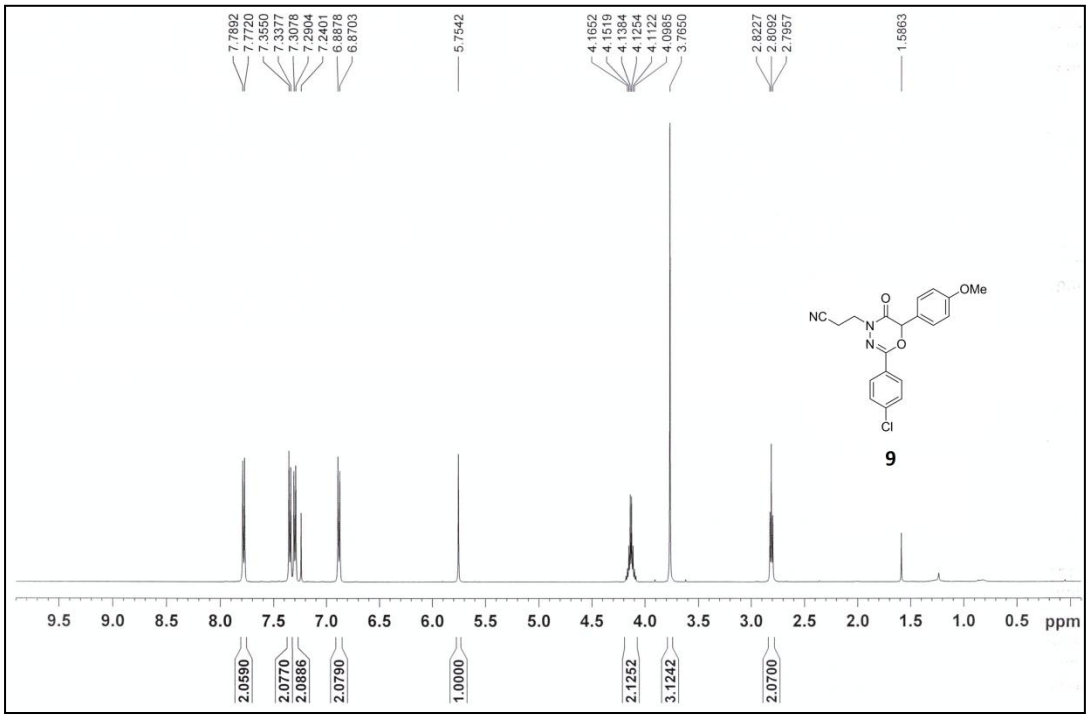
^{1}H NMR of compound **7** (300 MHz, $CDCl_3$)



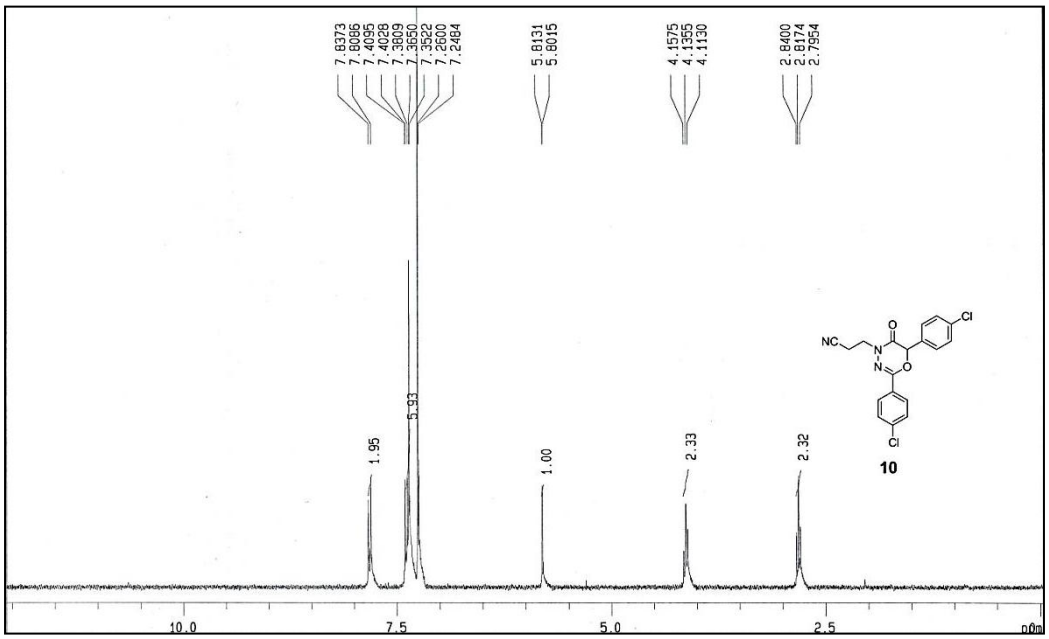
^{1}H NMR of compound **8** (300 MHz, $CDCl_3$)



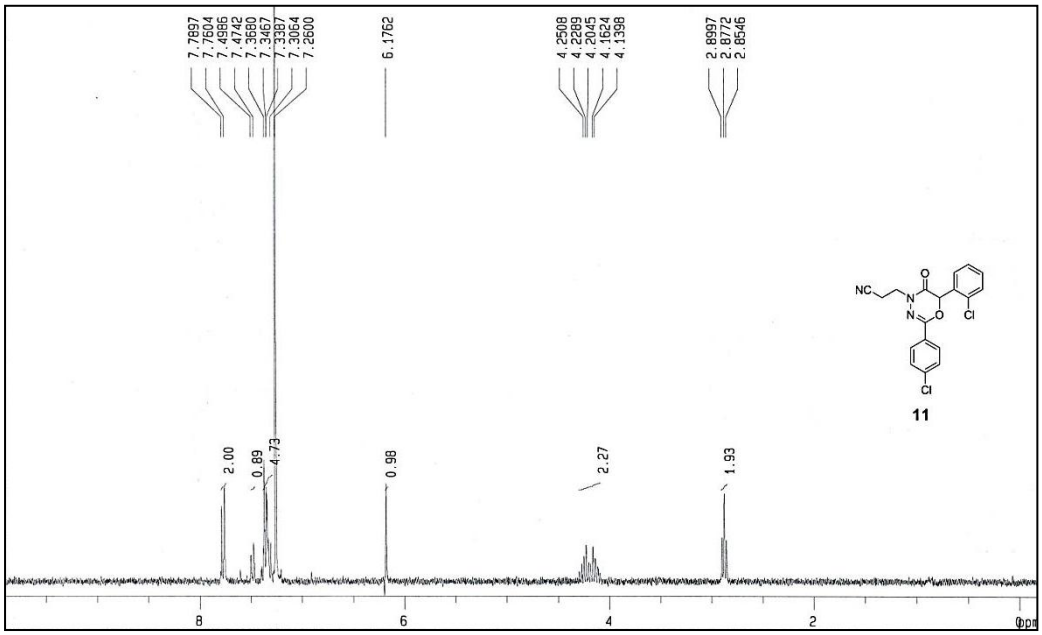
^{1}H NMR of compound **9** (500 MHz, $CDCl_3$)



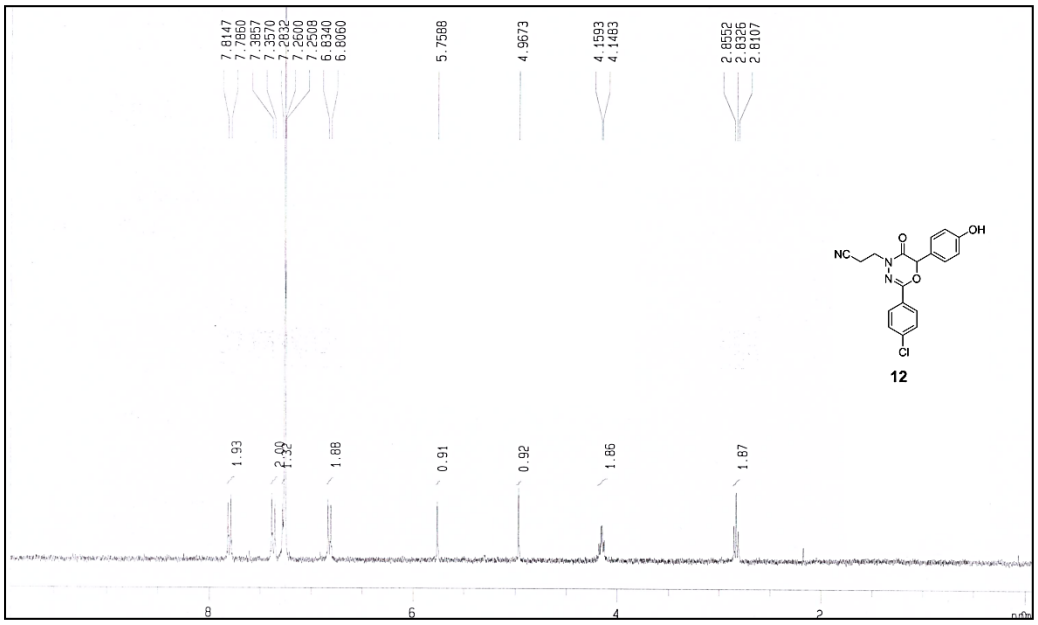
^{1}H NMR of compound **10** (300 MHz, $CDCl_3$)



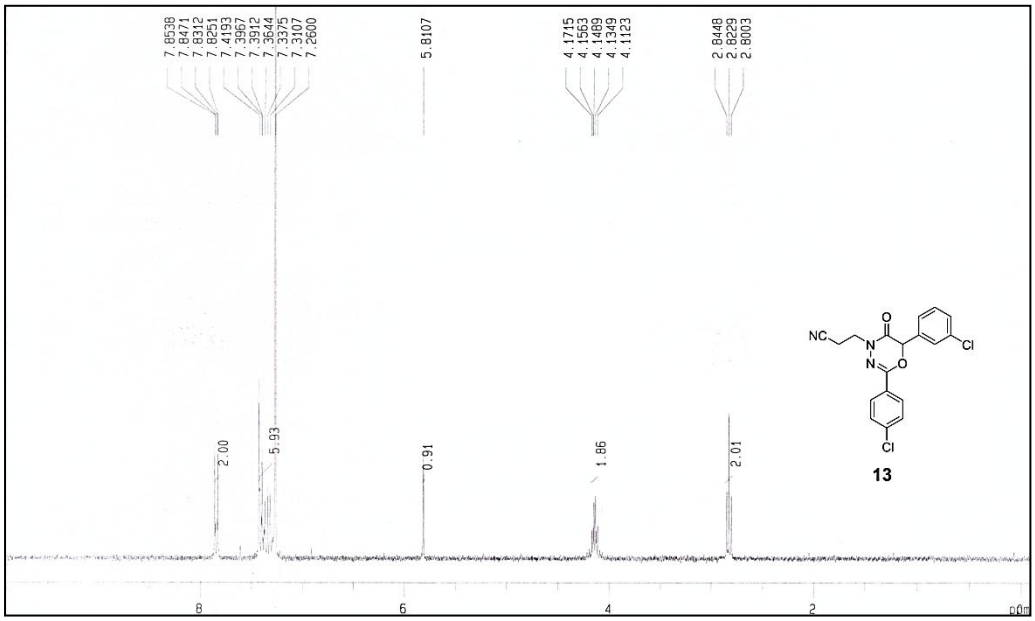
^{1}H NMR of compound **11** (300 MHz, $CDCl_3$)



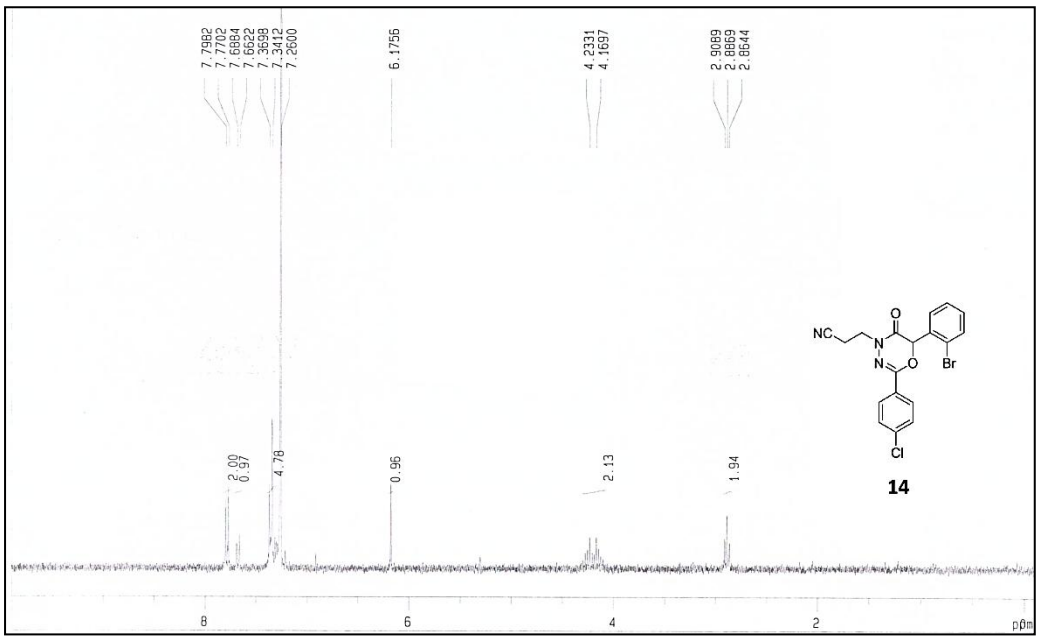
^{1}H NMR of compound **12** (300 MHz, $CDCl_3$)



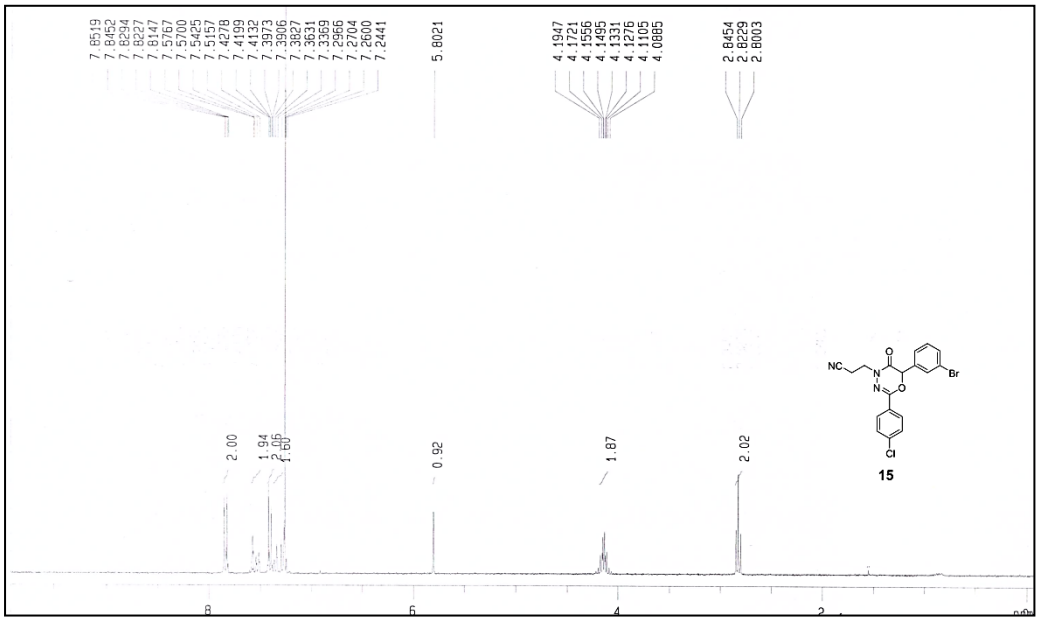
^{1}H NMR of compound **13** (300 MHz, $CDCl_3$)



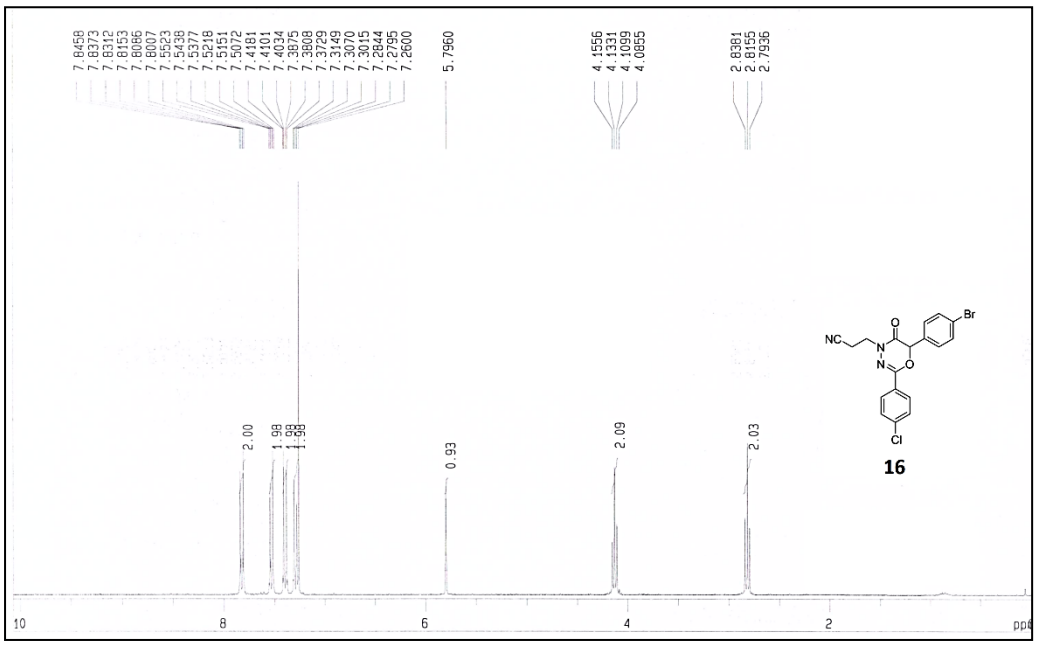
^{1}H NMR of compound **14** (300 MHz, $CDCl_3$)



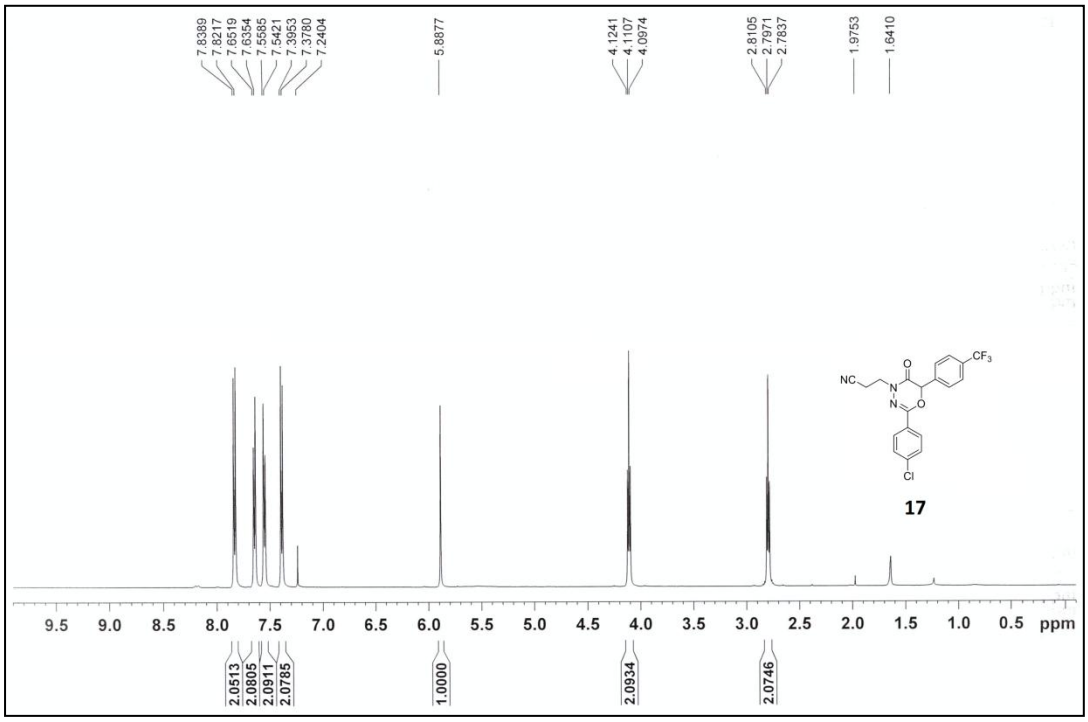
^{1}H NMR of compound **15** (300 MHz, $CDCl_3$)



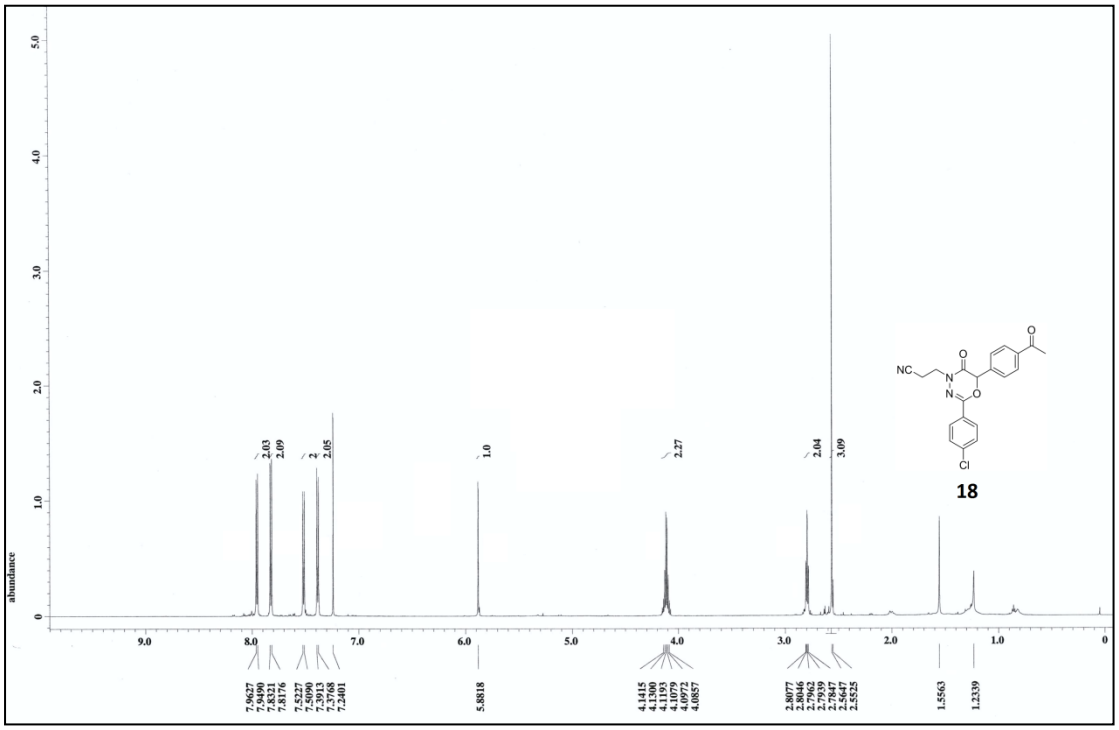
^{1}H NMR of compound **16** (300 MHz, $CDCl_3$)



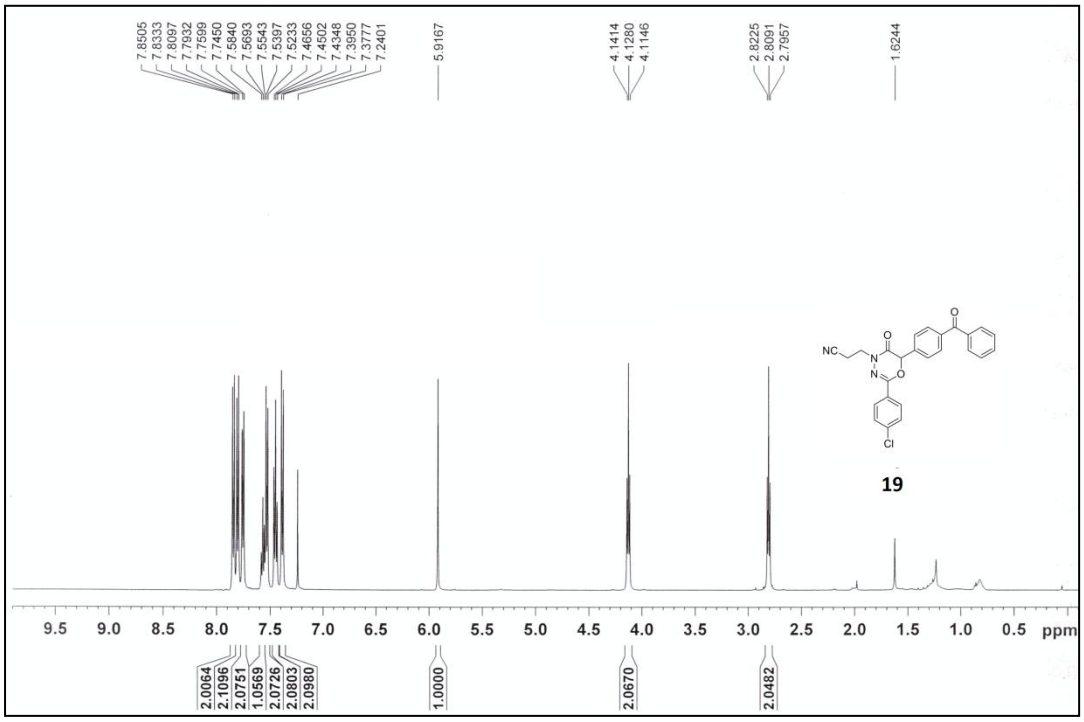
^{1}H NMR of compound **17** (500 MHz, $CDCl_3$)



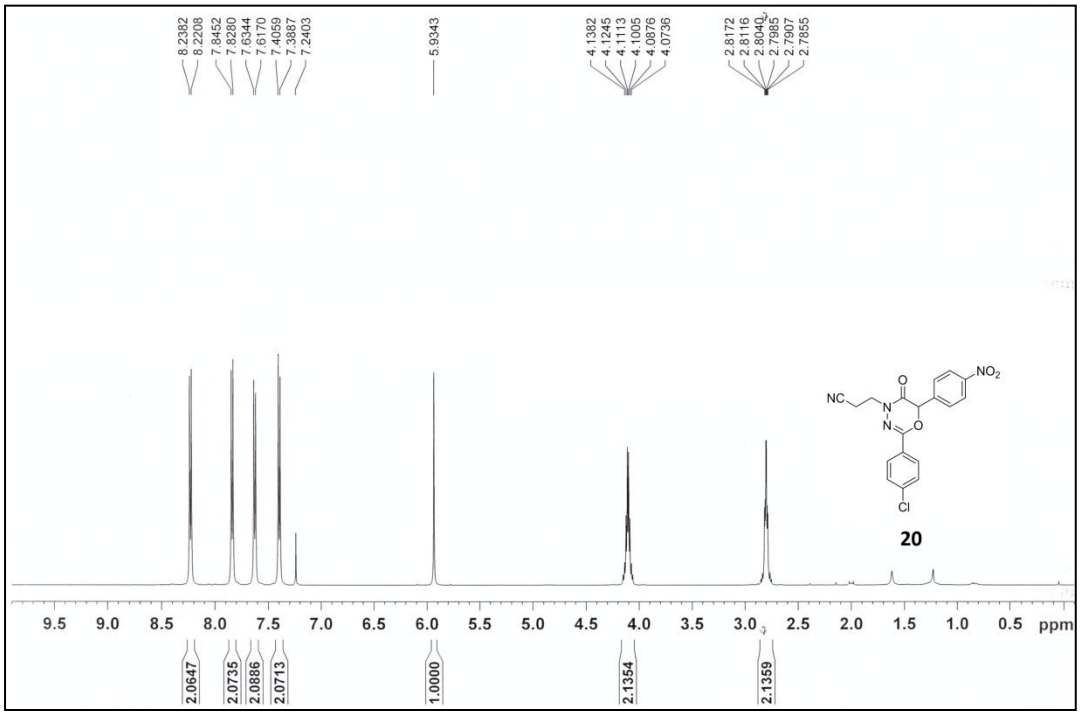
^{1}H NMR of compound **18** (600 MHz, $CDCl_3$)



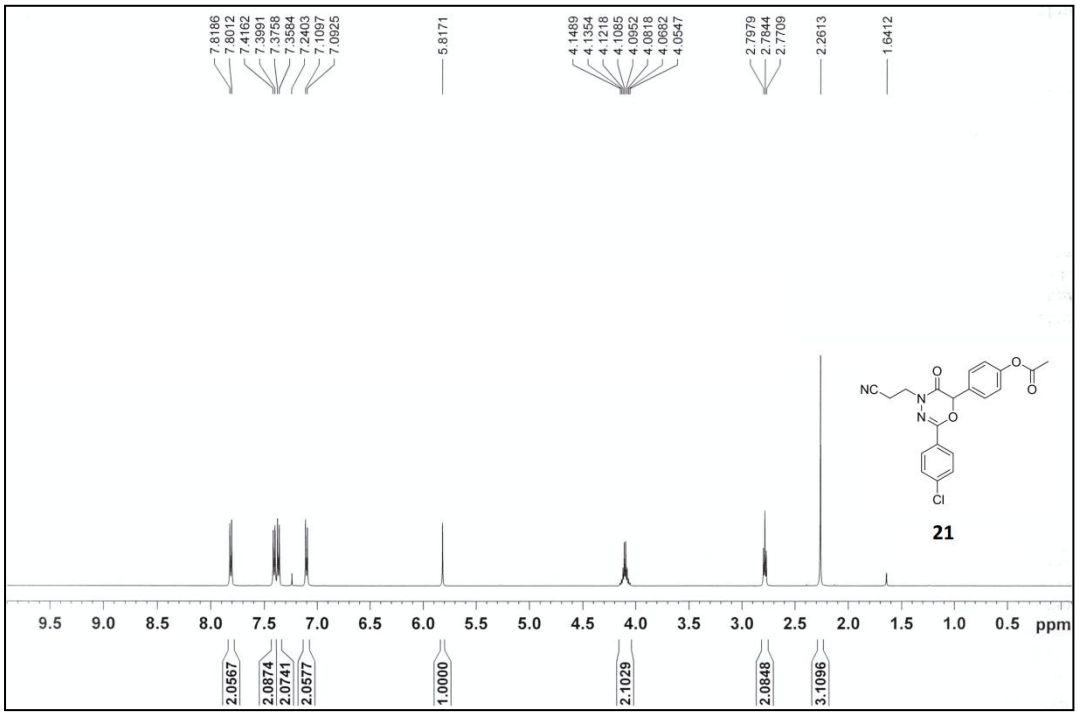
^{1}H NMR of compound **19** (500 MHz, $CDCl_3$)



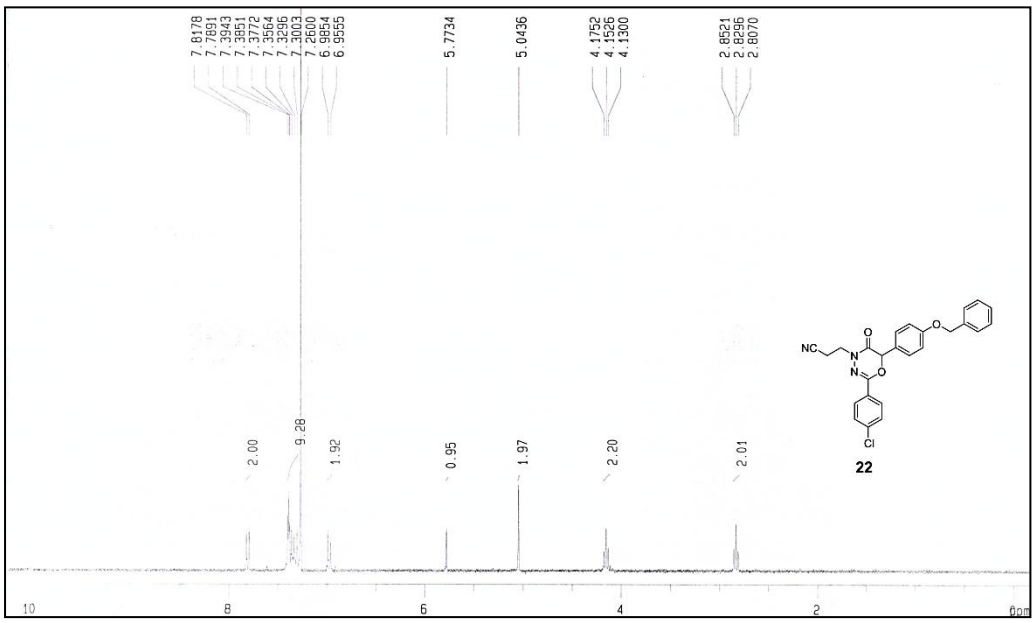
^{1}H NMR of compound **20** (500 MHz, $CDCl_3$)



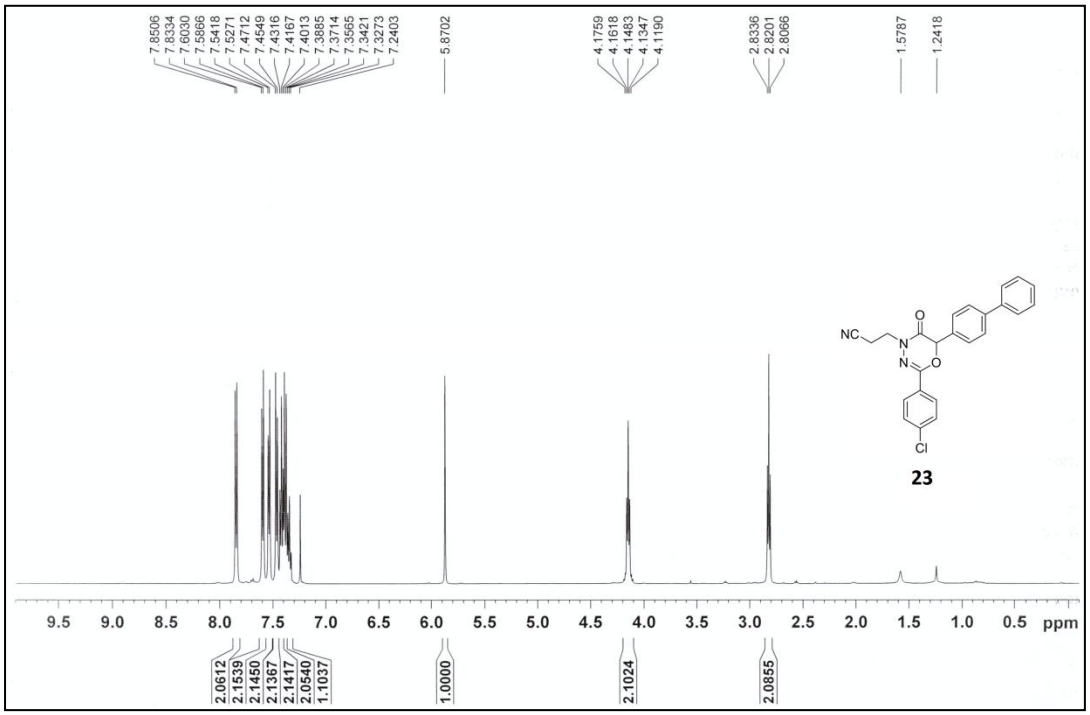
^{1}H NMR of compound **21** (500 MHz, $CDCl_3$)



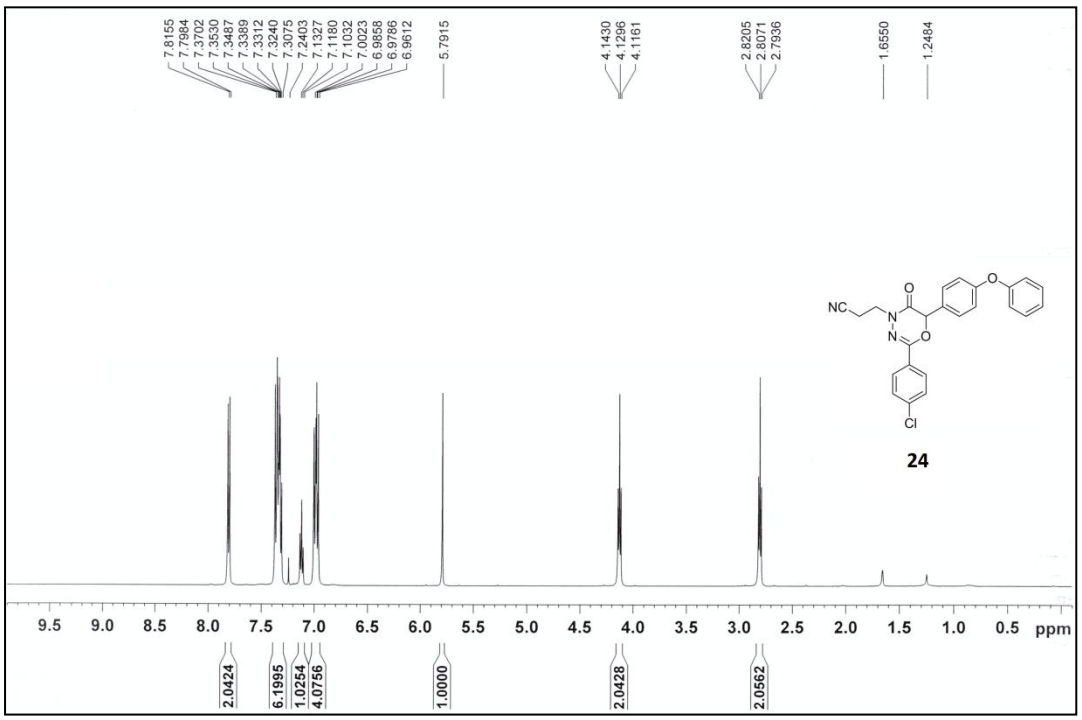
^{1}H NMR of compound **22** (300 MHz, $CDCl_3$)



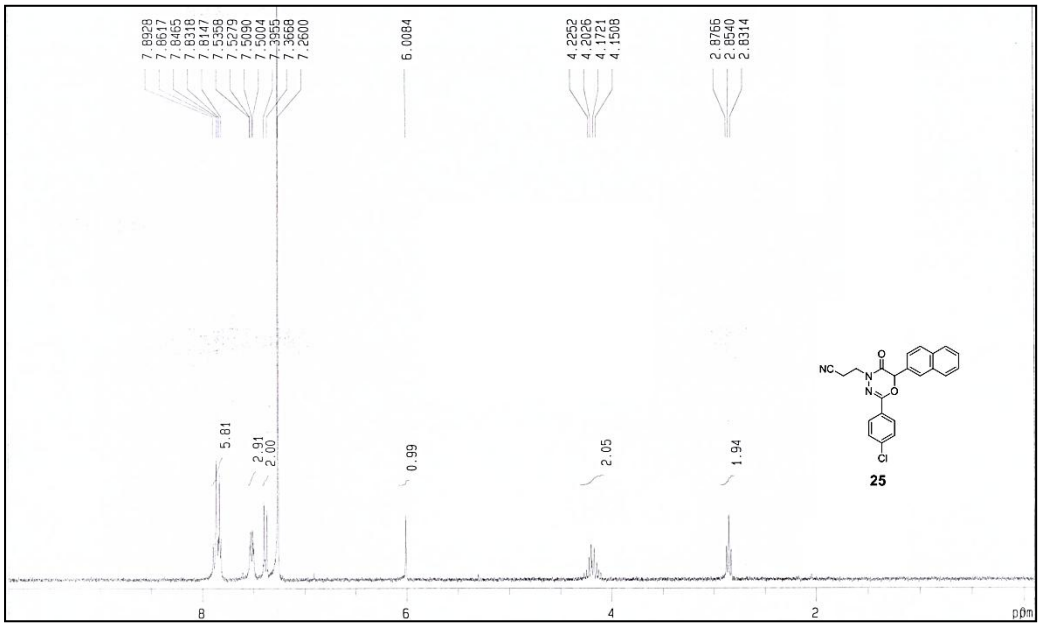
^{1}H NMR of compound **23** (500 MHz, $CDCl_3$)



^{1}H NMR of compound **24** (500 MHz, $CDCl_3$)

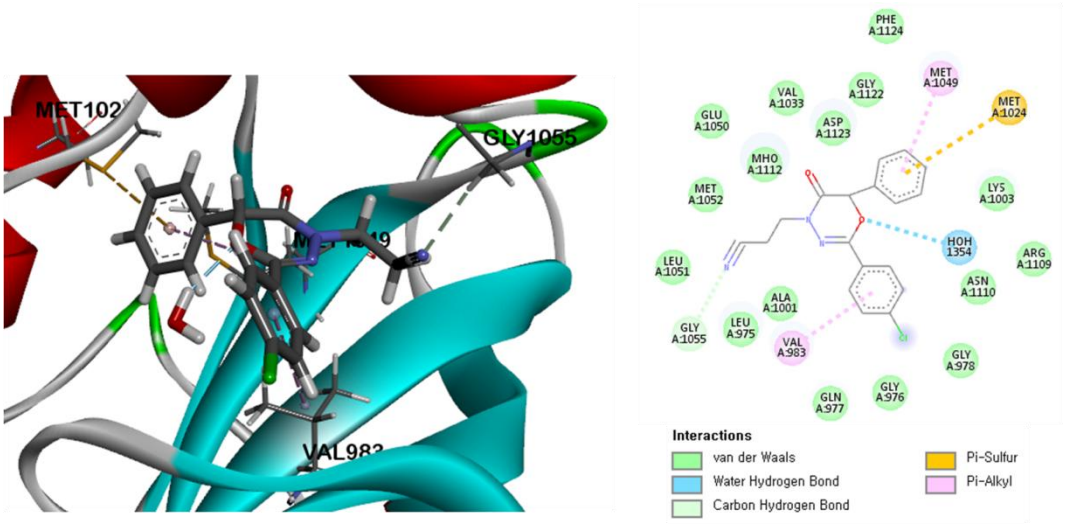


^{1}H NMR of compound **25** (300 MHz, $CDCl_3$)



5. Interaction between IGF1R (3D94, *Homo sapiens*) and compounds

LL-2003



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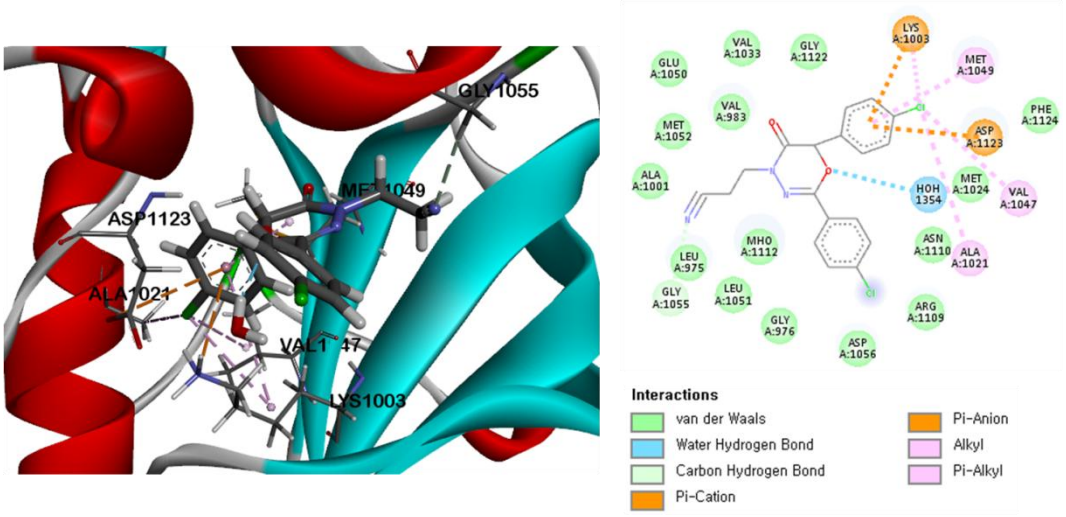

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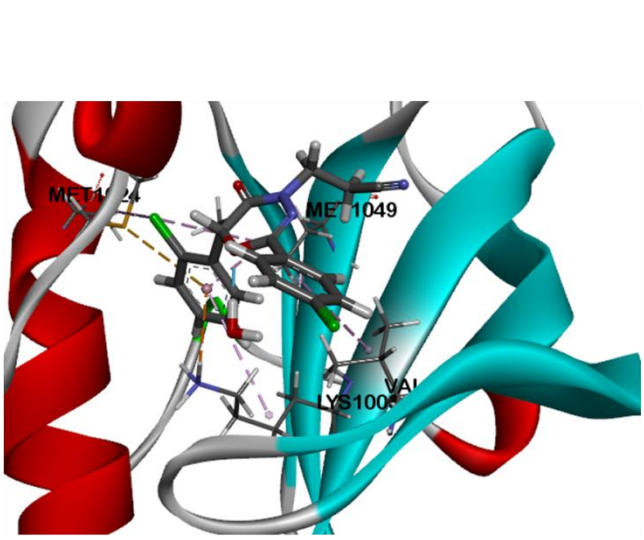
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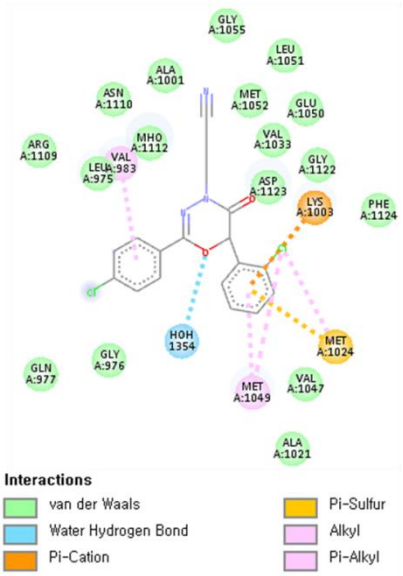
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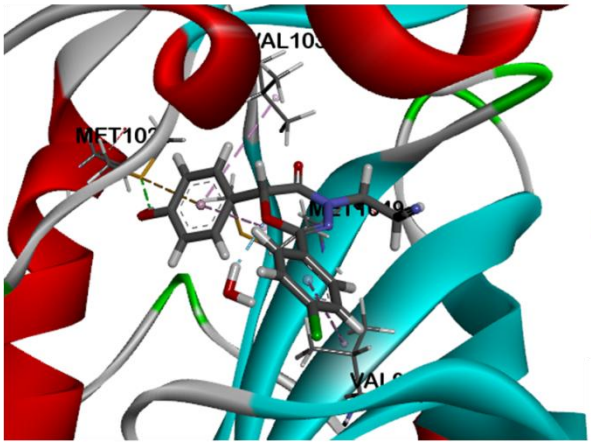
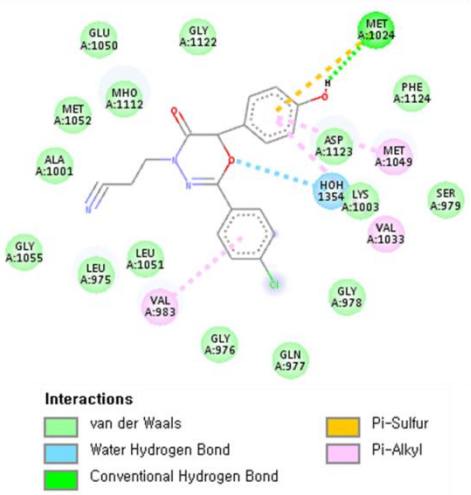
ALA A:1001
ASN A:1110
MET A:1052
GLU A:1050
ARG A:1109
MHO A:1112
VAL A:1033
VAL A:983
LEU A:975
GLY A:1122
ASP A:1123
LYS A:1003
PHE A:1124
HOH 1354
MET A:1024
GLY A:976
GLN A:977
MET A:1049
VAL A:1047
ALA A:1021
Interactions
van der Waals
Water Hydrogen Bond
Pi-Cation
Pi-Sulfur
Alkyl
Pi-Alkyl

12

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PHE A:1124
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ASP A:1123
MET A:1049
ALA A:1001
HOH 1354
LYS A:1003
SER A:979
VAL A:1033
GLY A:1055
LEU A:1051
LEU A:975
GLY A:978
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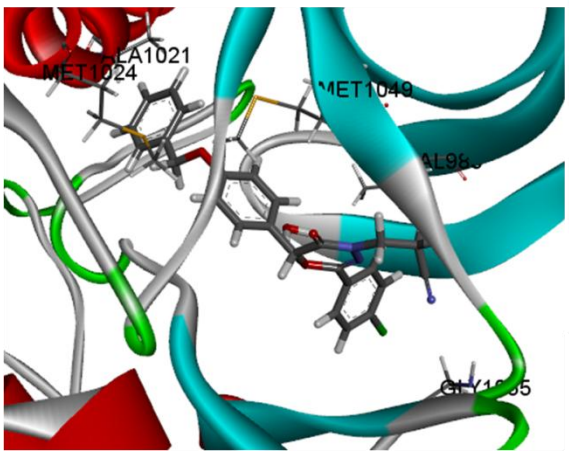
GLN A:977
Interactions
van der Waals
Water Hydrogen Bond
Conventional Hydrogen Bond
Pi-Sulfur
Pi-Alkyl

19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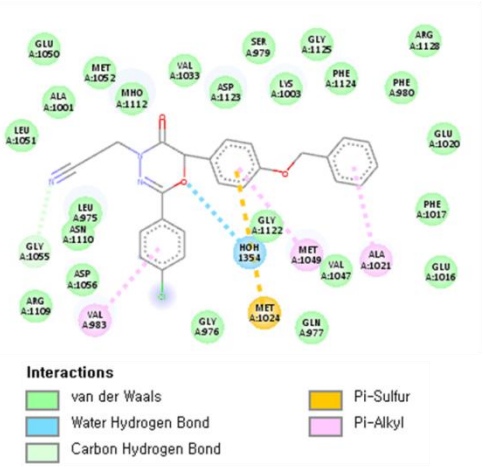
MET1049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PHE A:1017
GLY A:1122
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HOH 1354
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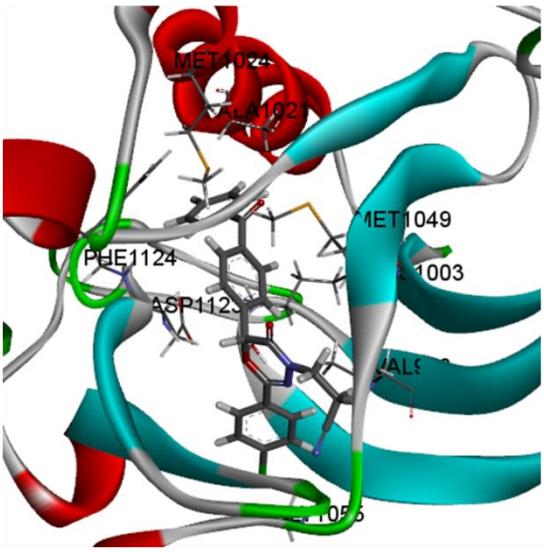
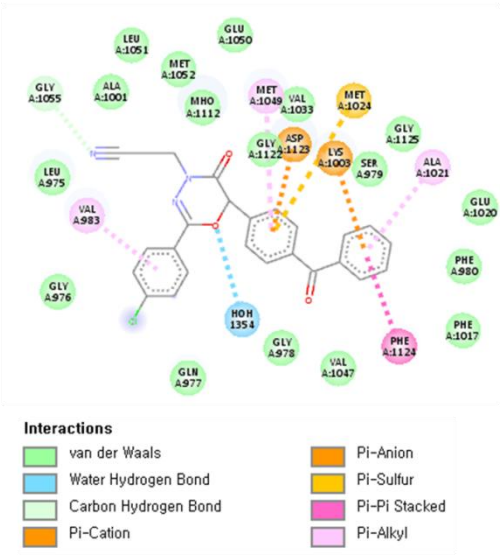
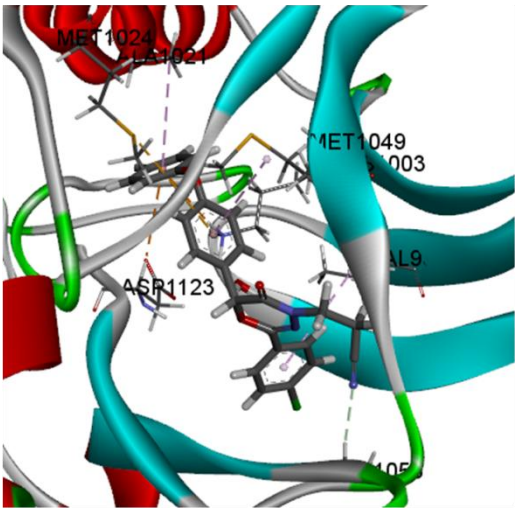
Interactions
van der Waals
Water Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sulfur
Pi-Alkyl

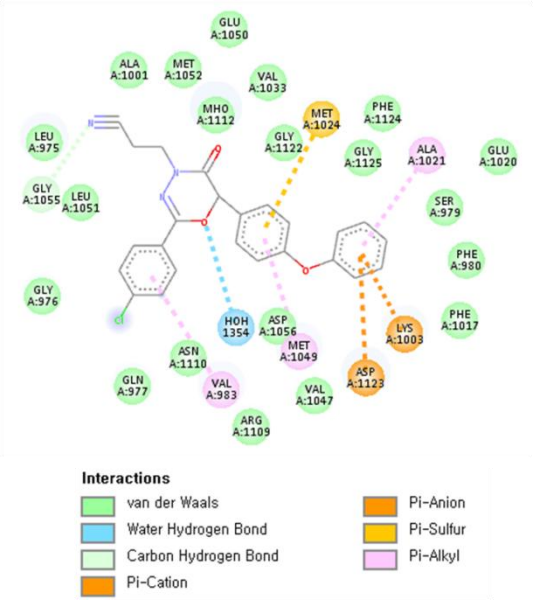
22


PHE1124

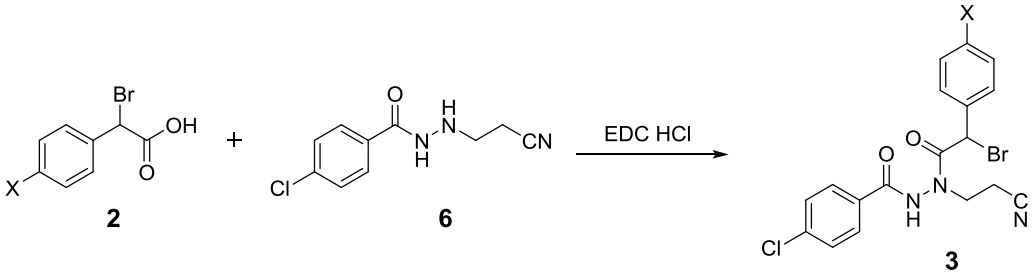

Interactions
van der Waals
Water Hydrogen Bond
Carbon Hydrogen Bond
Pi-Cation
Pi-Anion
Pi-Sulfur
Pi-Pi Stacked
Pi-Alkyl

24

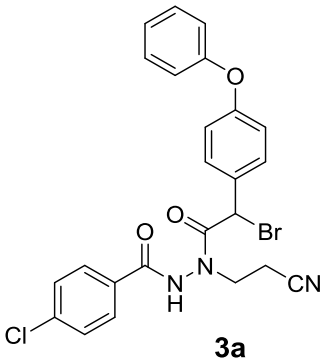

ASP1123


Interactions
van der Waals
Water Hydrogen Bond
Carbon Hydrogen Bond
Pi-Cation
Pi-Anion
Pi-Sulfur
Pi-Alkyl

6. [Part 2] Synthesis of 3a~3i



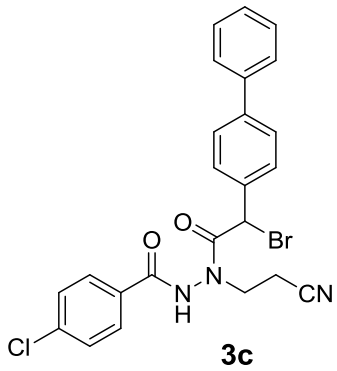
N'-(2-bromo-2-(4-phenoxyphenyl)acetyl)-4-chloro-N'-(2-cyanoethyl)benzo-hydrazide (3a)



A 25 mL round-bottom flask equipped with a stir bar was charged with compound **2a** (307 mg, 1.000 mmol, 1.00 eq.), compound **6** (224 mg, 1.699 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (1.5 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 1 hr and was observed by TLC. The organic phases were washed with brine (2 x 50 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford **3a** (309 mg, 60% yield) as a bright yellow caramel form.

R_f = 0.60 (EtOAc : hexane = 1 : 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.18 (br, 1H, amide-NH), 7.80-7.70 (m, 2H, acyl-CH), 7.52-7.47 (m, 2H, acyl-CH), 7.41-7.30 (m, 4H, aryl-CH), 7.15 (t, *J*= 7.5 Hz, 1H, aryl-CH), 7.02 (d, *J* = 7.5 Hz, 1H, aryl-CH), 6.97-6.92 (m, 2H, aryl-CH), 5.58 (s, 1H), 3.99 (br, 2H), 2.80 (br, 2H)

N'-(2-([1,1'-biphenyl]-4-yl)-2-bromoacetyl)-4-chloro-N'-(2-cyanoethyl)benzo-hydrazide (3c)

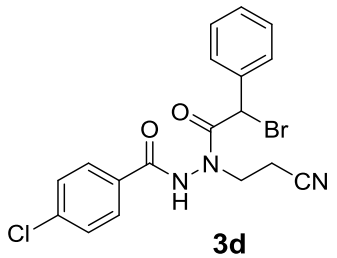


3c

A 50 mL round-bottom flask equipped with a stir bar was charged with compound **2c** (495 mg, 1.699 mmol, 1.00 eq.), compound **6** (380 mg, 1.699 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (1.50 eq.) and dichloromethane (20 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 75 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford **3c** (383 mg, 45% yield) as a bright yellow caramel form.

R_f = 0.71 (EtOAc : hexane = 1 : 1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.32 Hz, 2H, aryl-CH), 7.56-7.53 (m, 4H, aryl-CH), 7.49-7.40 (m, 6H, aryl-CH), 7.36 (t, *J* = 7.32 Hz, 1H, aryl-CH), 5.66 (s, 1H), 4.03 (br, 2H), 2.76 (br, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 169.7, 164.7, 142.3, 139.5, 129.5, 129.4 (4C), 128.9, 128.9 (4C), 128.5, 127.8, 127.6, 127.1 (4C), 118.4, 46.2, 16.3; ESI-MS *m/z* 496.00 $(M+H)^+$; HR-MS (FAB+) *m/z* 496.0421, (calculated for $C_{24}H_{20}O_2N_3BrCl^+$ $[M+H]^+$ 496.0427); HPLC flow rate = 1 mL/min, relative purity 66%, (t = 21.3 min)

N'-(2-bromo-2-phenylacetyl)-4-chloro-N'-(2-cyanoethyl)benzohydrazide (3d)



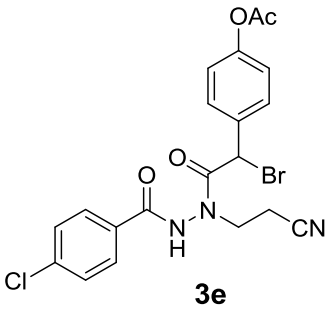
3d

A 100 mL round-bottom flask equipped with a stir bar was charged with compound **2d** (713 mg, 3.319 mmol, 1.00 eq.), compound **6** (742 mg, 3.319 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (1.50 eq.) and dichloromethane (35 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 100 mL), dried by Na_2SO_4 and evaporated to

give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford **3c** (710 mg, 51% yield) as a bright yellow caramel form.

R_f = 0.42 (EtOAc : hexane = 1 : 1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.05 (br, 1H, amide-NH), 7.71-7.65 (m, 2H, aryl-CH), 7.45-7.41 (m, 2H, aryl-CH), 7.37-7.36 (m, 1H, aryl-CH), 7.31-7.30 (m, 4H, aryl-CH), 5.67 (s, 1H), 3.95 (br, 2H), 2.72 (br, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.9, 164.9, 164.8, 139.7, 129.4 (2C), 129.3 (2C), 129.0 (2C), 128.9 (2C), 128.8, 128.0, 118.5, 46.1, 16.3; ESI-MS *m/z* 420.00 $(M+H)^+$; HR-MS (FAB+) *m/z* 420.0111, (calculated for $C_{18}H_{16}O_2N_3BrCl^+$ $[M+H]^+$ 420.0114); HPLC flow rate = 1 mL/min, relative purity 93%, (t = 18.2 min)

4-(1-bromo-2-(2-(4-chlorobenzoyl)-1-(2-cyanoethyl)hydrazinyl)-2-oxoethyl) phenyl acetate (3e)



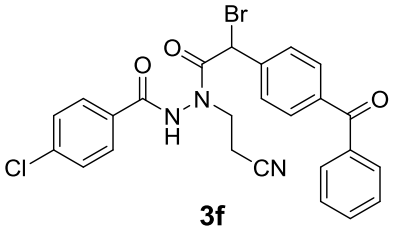
3e

A 100 mL round-bottom flask equipped with a stir bar was charged with compound **2e** (732 mg, 2.683 mmol, 1.00 eq.), compound **6** (600 mg, 2.683 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloromethane (30 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 100 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford **3e** (744 mg, 58% yield) as a bright yellow caramel form.

R_f = 0.41 (EtOAc : hexane = 1 : 1); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.61 (br, 1H, amide-NH), 7.67 (2H, *J* = 7.38 Hz, aryl-CH), 7.45 (2H, *J* = 8.7 Hz, aryl-CH), 7.35 (2H, *J* = 8.7 Hz, aryl-CH), 7.05 (d, 2H, *J* = 8.7 Hz, aryl-CH), 5.56 (s, 1H), 3.94 (br, 2H), 2.74 (br, 2H), 2.28 (s, 3H); ^{13}C-NMR (600 MHz, $CDCl_3$) δ 169.5 164.9, 151.3, 140.0, 139.9, 129.6, 129.5 (4C), 129.4, 129.0 (4C), 122.4, 118.5, 46.5, 21.2, 16.3; ESI-MS *m/z* 478.00 $(M+H)^+$; HR-MS (FAB+) *m/z* 478.0169 (calculated for

$C_{20}H_{18}O_4N_3BrCl^+$ $[M+H]^+$ 478.0169); HPLC flow rate = 1 mL/min, relative purity 70%, (t = 17.2 min)

N'-(2-(4-benzoylphenyl)-2-bromoacetyl)-4-chloro-N'-(2-cyanoethyl)benzo-hydrazide (3f)

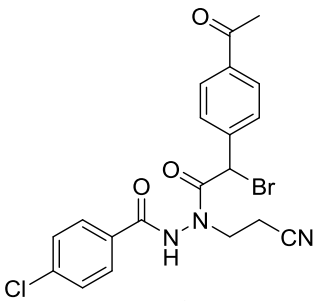


3f

A 50 mL round-bottom flask equipped with a stir bar was charged with compound **2f** (500 mg, 1.567 mmol, 1.00 eq.), compound **6** (350 mg, 1.567 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodi-imide HCl (1.50 eq.) and dichloromethane (20 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 75 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford **3f** (353 mg, 43% yield) as a bright yellow caramel form.

R_f = 0.33 (EtOAc : hexane = 1 : 1); ^1H NMR (800 MHz, $CDCl_3$) δ 9.41 (br, 1H, amide-NH), 7.77 (br, 2H , aryl-CH), 7.70-7.66 (m, 2H, aryl-CH), 7.59-7.57 (m, 1H, aryl-CH), 7.45-7.42 (m, 5H, aryl-CH), 5.72 (s, 1H), 4.20 (br, 1H), 3.66 (br, 1H), 2.72 (br, 2H); ^{13}C-NMR (200 MHz, $CDCl_3$) δ 196.4, 176.5, 169.4, 165.0, 139.8, 138.1, 136.8, 133.0, 130.4, 130.1 (2C), 129.4 (4C), 129.0 (4C), 128.4 (2C), 128.1, 118.3, 45.9, 16.3; ESI-MS *m/z* 524.10 $(M+H)^+$; HR-MS (FAB+) *m/z* 524.0378 (calculated for $C_{25}H_{20}O_3N_3BrCl^+$ $[M+H]^+$ 524.0377); HPLC flow rate = 1 mL/min, relative purity 100%, (t = 18.5 min)

N'-(2-(4-acetylphenyl)-2-bromoacetyl)-4-chloro-N'-(2-cyanoethyl)benzohydrazide (3g)

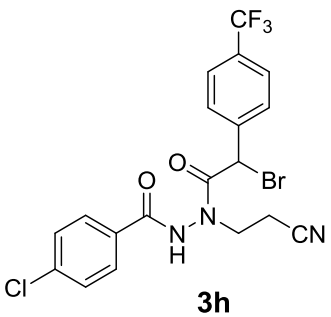


3g

A 100 mL round-bottom flask equipped with a stir bar was charged with compound **2g** (800 mg, 3.115 mmol, 1.00 eq.), compound **6** (697 mg, 3.115 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloromethane (35 mL). The reaction mixture was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 100 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford **3g** (515 mg, 36% yield) as a white solid.

R_f = 0.46 (EtOAc : hexane = 1 : 1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.00-7.77 (m, 4H, aryl-CH), 7.54-7.32 (m, 4H, aryl-CH), 5.98-5.93 (m, 1H), 4.14 (br, 1H), 3.74 (br, 1H), 2.79 (br, 2H), 2.55 (s, 3H); ^{13}C-NMR (75 MHz, CD_3OD) δ 200.5, 172.1, 171.8, 140.9, 132.0, 131.4 (4C), 130.8 (4C), 130.7, 130.6, 130.4, 120.0, 41.2, 27.6, 17.5; ESI-MS *m/z* 462.00 $(M+H)^+$; HR-MS (FAB+) *m/z* 462.0209 (calculated for $C_{20}H_{18}O_3N_3BrCl^+$ $[M+H]^+$ 462.0220); HPLC flow rate = 1 mL/min, relative purity 50%, (t = 17.0 min)

N'-(2-bromo-2-(4-(trifluoromethyl)phenyl)acetyl)-4-chloro-N'-(2-cyanoethyl)benzohydrazide (3h)



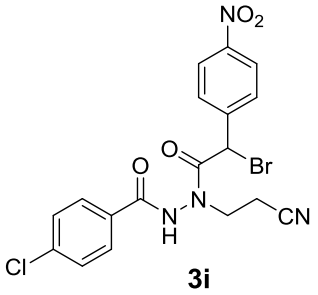
3h

A 100 mL round-bottom flask equipped with a stir bar was charged with compound **2h** (800 mg, 2.827 mmol, 1.00 eq.), compound **6** (632 mg, 2.827 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloromethane (30 mL). The reaction mixture

was stirred at room temperature during 2 hr and was observed by TLC. The organic phases were washed with brine (2 x 100 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 2) to afford **3h** (807 mg, 59% yield) as a bright yellow caramel form.

R_f = 0.59 (EtOAc/hexane = 1/1); ^{1}H NMR (800 MHz, $CDCl_3$) δ 9.13 (br, 1H, amide-NH), 7.75-7.70 (m, 2H, aryl-CH), 7.55-7.54 (m, 2H, aryl-CH), 7.52-7.51 (m, 2H, aryl-CH), 7.47-7.44 (m, 2H, aryl-CH), 5.65-5.62 (m, 1H), 4.26 (br, 1H), 3.58 (br, 1H), 2.74 (br, 2H); ^{13}C-NMR (200 MHz, $CDCl_3$) δ 165.0, 164.9, 131.5-131.0 (m), 129.4 (2C), 129.5 (2C), 129.4 (2C), 128.9 (4C), 128.6, 125.6, 125.7-121.6 (q, J=270.7 Hz), 118.5, 45.9, 16.3; ESI-MS *m/z* 488.00 $(M+H)^+$; HR-MS (FAB+) *m/z* 487.9996 (calculated for $C_{19}H_{15}O_2N_3BrClF_3^+$ $[M+H]^+$ 487.9988); HPLC flow rate = 1 mL/min, relative purity 56%, (t = 18.4 min)

N'-(2-bromo-2-(4-nitrophenyl)acetyl)-4-chloro-N'-(2-cyanoethyl)benzohydrazide (3i)



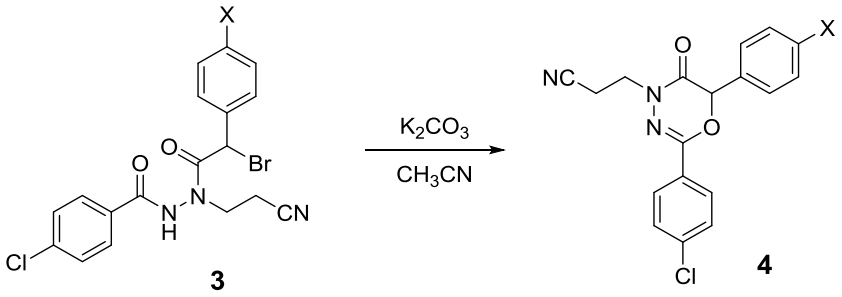
3i

A 100 mL round-bottom flask equipped with a stir bar was charged with compound **2i** (516 mg, 2.307 mmol, 1.00 eq.), compound **6** (600 mg, 2.307 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (1.5 eq.) and dichloromethane : EtOAc = 1 : 1 (30 mL). The reaction mixture was stirred at room temperature during 2hr and was observed by TLC. The combined organic phases were washed with brine (2 x 100 mL), dried by Na_2SO_4 and evaporated to give the crude product. This was purified by by silica gel flash chromatography (EtOAc : Hexane = 1: 2) to afford **3i** (619 mg, 58% yield) as white solid.

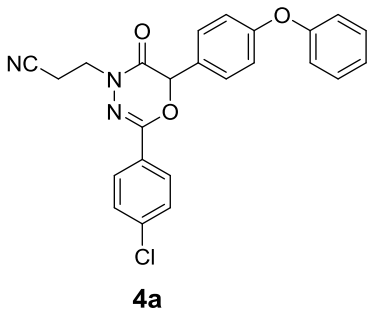
R_f = 0.32 EtOAc : hexane = 1 : 1; ^{1}H NMR (300 MHz, CD_3OD) δ 8.30-8.13 (m, 2H, aryl-CH), 7.86-7.80 (m, 2H, aryl-CH), 7.69 (br, 2H, aryl-CH), 7.57-7.52 (m, 2H, aryl-CH), 6.03 (s, 1H), 4.13 (br, 1H), 3.78 (br, 1H), 2.78 (br, 2H); ^{13}C-NMR (75 MHz,

CD_3OD) δ 168.7, 150.2, 141.0, 132.3, 131.6, 131.5 (2C), 131.4 (2C), 130.9 (4C), 125.7, 125.3, 120.0, 47.2, 17.4; ESI-MS *m/z* 465.00 $(M+H)^+$; HR-MS (FAB+) *m/z* 464.9952, (calculated for $C_{18}H_{15}O_4N_4BrCl^+$ $[M+H]^+$ 464.9965); HPLC flow rate = 1 mL/min, relative purity 70%, (t = 17.9 min)

7. [Part 2] Synthesis of 4a~4i



3-(2-(4-chlorophenyl)-5-oxo-6-(4-phenoxyphenyl)-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (4a)

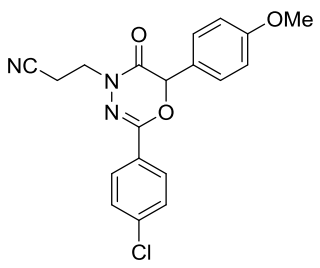


A 50 mL round-bottom flask equipped with a stir bar was charged with compound **3a** (110 mg, 0.2149 mmol, 1.00 eq.), acetonitrile (15 mL) and K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature, 50 ºC and 75 ºC during 8 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **4a** (81 mg, ,88% yield) as white solid.

R_f = 0.63 (EtOAc : hexane = 1 : 2);); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.55 Hz, 2H, aryl-CH), 7.37-7.31 (m, 6H, aryl-CH), 7.12 (t, *J* = 7.35 Hz, 1H, aryl-CH), 6.99 (2H, *J* = 8.25 Hz, 2H, aryl-CH), 6.97 (2H, *J* = 8.7 Hz, 2H, aryl-CH), 5.79 (s, 1H), 4.13 (t, 2H), 2.81 (t, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 159.9, 158.8, 156.0, 148.1,

137.5, 129.8 (2C), 128.8 (2C), 128.6, 128.4 (2C), 128.0 (2C), 127.7, 124.0 (2C), 119.6 (2C), 118.6 (2C), 117.2, 76.6, 43.4, 16.4; ESI-MS *m/z* 432.10 $(M+H)^+$; HR-MS (FAB+) *m/z* 432.1119 (calculated for $C_{24}H_{19}O_3N_3Cl^+$ $[M+H]^+$ 432.1115); FT-IR (KBr): 2994, 2357, 1770, 1685, 1636, 1589, 1507, 1489, 1403, 1241, 1170, 1091, 1013, 873, 838, 755, 693 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 15.4 min)

3-(2-(4-chlorophenyl)-5-oxo-6-(4-methoxyphenyl)-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (4b)

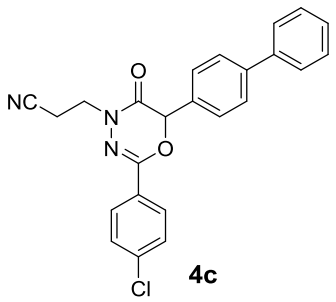


4b

A 25 mL round-bottom flask equipped with a stir bar was charged with compound **2b** (189 mg, 0.772 mmol, 1.00 eq.), compound **6** (173 mg, 0.772 mmol, 1.00 eq.), 1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (1.50 eq.) and dichloromethane (10 mL). The reaction mixture was stirred at room temperature during 1 hr and was observed by TLC. The organic phases were washed with brine (2 x 50 mL), dried by Na_2SO4 and evaporated to give the crude product. This was purified by silica gel flash chromatography (EtOAc : Hexane = 1 : 3) to afford **3c** (30 mg, 11% yield) as a bright yellow caramel form.

R_f = 0.62 (EtOAc : hexane = 1 : 1); ^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (t, *J* = 8.6 Hz, 2H, aryl-CH), 7.34 (d, *J* = 8.65 Hz, 2H, aryl-CH), 7.30 (d, *J* = 8.7 Hz, 2H, aryl-CH), 6.88 (d, *J* = 8.75 Hz, 2H, aryl-CH), 5.75 (s, 1H), 4.17-4.10 (m, 2H), 3.77 (s, 3H), 2.81 (t, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 160.6, 160.1, 148.3, 137.5, 128.8 (2C), 128.3 (2C), 128.1 (2C), 127.9, 126.4, 117.2, 114.5 (2C), 76.8, 55.3, 43.4, 16.5; ESI-MS *m/z* 370.10 $(M+H)^+$; HR-MS (FAB+) *m/z* 370.0961 (calculated for $C_{19}H_{17}O_3N_3Cl^+$ $[M+H]^+$ 370.0958); FT-IR (KBr): 2959, 2838, 2251, 1685, 1636, 1611, 1514, 1493, 1441, 1403, 1352, 1328, 1307, 1250, 1178, 1109, 1091, 1031, 1014, 838, 791, 774, 722, 677, 647, 630 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 19.2 min)

3-(6-([1,1'-biphenyl]-4-yl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (4c)

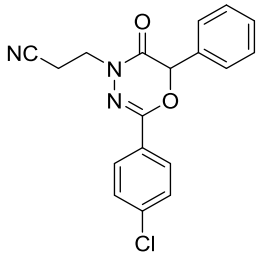


4c

A 50 mL round-bottom flask equipped with a stir bar was charged with compound **3c** (130 mg, 0.2617 mmol, 1.00 eq.), acetonitrile (15 mL) and K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature, 50 ºC and 75 ºC during 8 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **4c** (91 mg, 86% yield) as white solid.

R_f = 0.70 (EtOAc : hexane = 1 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.6 Hz, 2H, aryl-CH), 7.59 (d, *J* = 8.2 Hz, 2H, aryl-CH), 7.53 (d, *J* = 7.35 Hz, 2H, aryl-CH), 7.46 (d, *J* = 8.15 Hz, 2H, aryl-CH), 7.42 (t, *J* = 7.45 Hz, 2H, aryl-CH), 7.36 (d, *J* = 7.45 Hz, 2H, aryl-CH), 7.34 (t, *J* = 7.2 Hz, 1H, aryl-CH), 5.87 (s, 1H), 4.18-4.12 (t, 2H), 2.82 (t, 2H); 13C-NMR (125 MHz, $CDCl_3$) δ 159.8, 148.2, 142.6, 140.1, 137.6, 133.1, 128.8 (2C), 128.1 (2C), 127.81 (2C), 127.75 (2C), 127.10 (2C), 127.08 (2C), 117.2, 76.9, 43.5, 16.5; ESI-MS *m/z* 416.10 $(M+H)^+$; HR-MS (FAB+) *m/z* 416.1176 (calculated for $C_{24}H_{19}O_2N_3Cl^+$ $[M+H]^+$ 416.1166); FT-IR(KBr) 3032, 2310, 1685, 1638, 1599, 1491, 1403, 1248, 1162, 1092, 1014, 839, 757, 736, 697 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 21.1 min)

3-(2-(4-chlorophenyl)-5-oxo-6-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (4d)

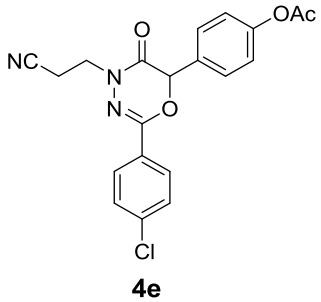


4d

A 50 mL round-bottom flask equipped with a stir bar was charged with compound **3d** (185 mg, 0.4395 mmol, 1.00 eq.), acetonitrile (15 mL) and K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature, 50 ºC and 75 ºC during 8 hours and was observed by TLC then cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **4d** (136.7mg, 91% yield) as white solid.

R_f = 0.53 (EtOAc : hexane = 1 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.6 Hz, 2H, aryl-CH), 7.35-7.39 (m, 7H, aryl-CH), 5.82 (s, 1H), 4.12 (t, 2H), 2.80 (t, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 159.8, 148.1, 137.5, 134.3, 129.6, 129.1(2C), 128.8 (2C), 128.0 (2C), 127.8, 126.5 (2C), 117.2, 77.0, 43.4, 16.4; ESI-MS *m/z* 340.10 $(M+H)^+$ 340.0859 (calculated for $C_{18}H_{15}O_2N_3Cl^+$ $[M+H]^+$ 340.0853); FT-IR (KBr) 3065, 2956, 2309, 2251, 1685, 1638, 1599, 1492, 1403, 1361, 1330, 1270, 1197, 1163, 1108, 1092, 1014, 839, 758, 722, 699, 616 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 19.6 min)

4-(2-(4-chlorophenyl)-4-(2-cyanoethyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-6-yl)phenyl acetate (4e)



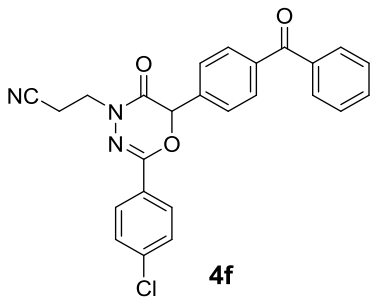
4e

A 50 mL round-bottom flask equipped with a stir bar was charged with compound **3e** (264 mg, 0.5517 mmol, 1.00 eq.), acetonitrile (20 mL) and K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature, 50 ºC and 75 ºC during 8 hours and was observed by TLC then

cooled to room temperature. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **4e** (136.7mg, 91% yield) as white solid.

R_f = 0.32 (EtOAc : hexane = 1 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.7 Hz , 2H, aryl-CH), 7.41 (d, *J* = 8.55 Hz, 2H, aryl-CH), 7.37 (d, *J* = 8.7 Hz, 2H, aryl-CH), 7.10 (d, *J* = 8.6 Hz), 2H, aryl-CH), 5.82 (s, 1H), 4.15-4.05 (m, 2H), 2.78 (t, 2H), 2.26 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.0, 159.5, 151.5, 147.9, 131.7, 128.8 (2C), 128.0 (2C), 127.8 (2C), 127.6, 122.3 (2C), 117.1, 76.4, 43.4, 21.0, 16.4; ESI-MS *m/z* 398.10 $(M+H)^+$; HR-MS (FAB+) *m/z* 398.0904 (calculated for $C_{20}H_{17}O_4N_3Cl^+$ $[M+H]^+$ 398.0908.); FT-IR (KBr) 2349, 2309, 1761, 1685, 1638, 1599, 1508, 1493, 1403, 1369, 1201, 1169, 1092, 1014, 912, 840, 773, 721, 671, 654 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 19.1 min)

3-(6-(4-benzoylphenyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (4f)



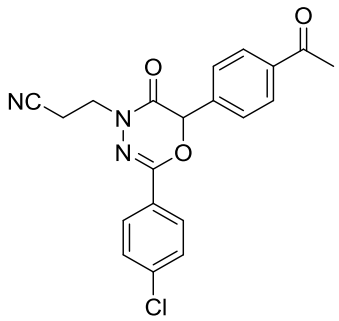
4f

A 25 mL round-bottom flask equipped with a stir bar was charged with compound **3f** (125 mg, 0.239 mmol, 1.00 eq.), acetonitrile (10 mL) and K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature during 8 hours and was observed by TLC. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **4f** (80 mg, 75% yield) as white solid.

R_f = 0.42 (EtOAc : hexane = 1 : 2), R_f = 0.43 (EtOAc : hexane : CH_2Cl_2 = 1: 2 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.6 Hz, 2H, aryl-CH) , 7.80 (d, *J* = 8.25 Hz, 2H, aryl-CH), 7.75 (d, *J* = 7.45 Hz, 2H, aryl-CH), 7.57 (t, *J* = 7.45 Hz, 1H, aryl-CH), 7.53

(d, J = 8.2 Hz, 2H, aryl-CH), 7.45 (t, J = 7.7 Hz, 2H, aryl-CH), 7.39 (d, J = 8.65 Hz, 2H, aryl-CH, (5.92 (s, 1H), 4.13 (t, 2H), 2.81 (t, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 195.8, 159.2, 148.0, 138.6, 138.1, 137.7, 137.0, 132.7, 130.6 (2C), 130.0 (2C), 128.9 (2C), 128.4 (2C), 128.0 (2C), 127.4, 126.4 (2C), 117.1, 76.5, 43.4, 16.4; ESI-MS *m/z* 444.10 $(M+H)^+$; HR-MS (FAB+) *m/z* 444.1123 (calculated for $C_{25}H_{19}O_3N_3Cl^+$ $[M+H]^+$ 444.1115); FT-IR (KBr) 3726, 3706, 3625, 3063, 2382, 2360, 2349, 2308, 2251, 1686, 1659, 1598, 1492, 1446, 1402, 1351, 1317, 1279, 1162, 1109, 1091, 1014, 939, 926, 839, 773, 720, 701, 677, 649 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 20.2 min)

3-(6-(4-acetylphenyl)-2-(4-chlorophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (4g)



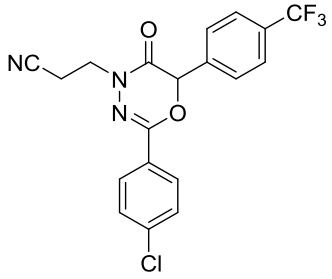
4g

A 50 mL round-bottom flask equipped with a stir bar was charged with compound **3g** (188 mg, 0.401 mmol, 1.00 eq.), acetonitrile (20 mL) and K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature during 8 hours and was observed by TLC. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane : CH_2Cl_2 = 1 : 3 : 1) to afford **4g** (146.1 mg, 94% yield) as white solid.

R_f = 0.33 (EtOAc : hexane = 1 : 2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 (d, J = 6.85 Hz, 2H, aryl-CH), 7.82 (d, J = 7.25 Hz, 2H, aryl-CH), 7.52 (d, J = 6.85 Hz, 2H, aryl-CH), 7.38 (d, J = 8.7 Hz, 2H, aryl-CH), 5.88 (s, 1H), 4.14-4.09 (m, 2H), 2.81-2.78 (m, 2H), 2.56 (s, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 197.2, 159.2, 148.0, 138.8, 137.9, 137.8, 129.0 (4C), 128.5 (2C), 127.5, 126.8 (2C), 117.0, 76.5, 43.5, 26.7, 16.4; ESI-MS *m/z* 382.10 $(M+H)^+$; HR-MS (FAB+) *m/z* 382.0945 (calculated for $C_{20}H_{17}O_3N_3Cl^+$ $[M+H]^+$ 382.0958); FT-IR (KBr) 3726, 3625, 2349, 2308, 1685, 1640, 1491, 1402,

1362, 1267, 1091, 1013, 838, 720, 679, 649 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 18.9 min)

3-(2-(4-chlorophenyl)-5-oxo-6-(4-trifluoromethylphenyl)-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (4h)

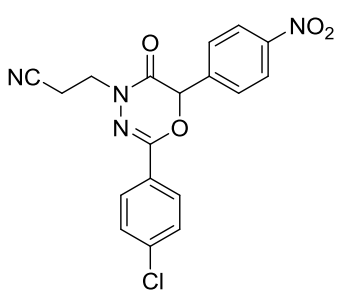


4h

A 100 mL round-bottom flask equipped with a stir bar was charged with compound **3h** (298 mg, 0.6090 mmol, 1.00 eq.), acetonitrile (30 mL) and K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature during 8 hours and was observed by TLC. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 3) to afford **4h** (185 mg, 75% yield) as white solid.

R_f = 0.52 (EtOAc : hexane = 1 : 2); ^1H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.6 Hz, 2H, aryl-CH), 7.64 (d, *J* = 8.25 Hz, 2H, aryl-CH), 7.55 (d, *J* = 8.2 Hz, 2H, aryl-CH), 7.39 (d, *J* = 8.65 Hz, 2H ,aryl-CH), 5.89 (s, 1H), 4.11 (t, 2H), 2.80 (t, 2H); 13C-NMR (125 MHz, $CDCl_3$) δ 159.1, 147.9, 137.9, 137.8, 131.8-131.5 (q, *J* = 32.625 Hz), 128.9 (2C), 128.0 (2C), 127.3, 126.9 (2C), 126.0 (2C, d, *J* = 3.5 Hz), 124.7-122.6 (d, *J* = 271 Hz), 117.1, 76.3, 43.4, 16.4; ESI-MS *m/z* 408.10 $(M+H)^+$; HR-MS (FAB+) *m/z* 407.0644 (calculated for $C_{19}H_{13}O_2N_3F_3Cl^+$ $[M+H]^+$ 407.0648); FT-IR (KBr) 3725, 3624, 2381, 2359, 2349, 2308, 1487, 1640, 1493, 1404, 1326, 1167, 1126, 1092, 1067, 1015, 839, 778, 720, 672, 649 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 20.2 min)

3-(2-(4-chlorophenyl)-6-(4-nitrophenyl)-5-oxo-5,6-dihydro-4H-1,3,4-oxadiazin-4-yl)propanenitrile (4i)

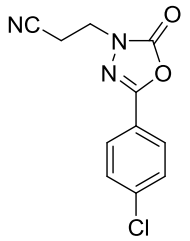


4i

A 50 mL round-bottom flask equipped with a stir bar was charged with compound **3d** (250 mg, 0.5368 mmol, 1.00 eq.), anhydrous acetonitrile (20 mL) and K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature until full conversion and was observed by TLC. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : hexane = 1 : 2) to afford **4d** (140mg, 68% yield) as white solid.

R_f = 0.39 (EtOAc : hexane = 1 : 2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.7 Hz, 2H, aryl-CH), 7.84 (d, *J* = 8.6 Hz, 2H, aryl-CH), 7.63 (d, *J* = 8.7 Hz, 2H, aryl-CH), 7.40 (d, *J* = 8.6 Hz, 2H, aryl-CH), 5.93 (s, 1H), 4.14-4.07 (m, 2H), 2.82-2.79 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 158.6, 148.5, 147.7, 140.6, 137.9, 129.0 (2C), 127.9 (2C), 127.6 (2C), 127.1, 124.1 (2C), 117.0, 75.9, 43.4, 16.4; ESI-MS *m/z* 385.09 $(M+H)^+$; HR-MS (FAB+) *m/z* 385.0700, (calculated for $C_{18}H_{14}O_4N_4Cl^+$ $[M+H]^+$ 385.0704); FT-IR (KBr) 3726, 3080, 2349, 2308, 2251, 1686, 1639, 1600, 1524, 1493, 1403, 1349, 1271, 1161, 1110, 1092, 1014, 839, 735, 721, 694, 652 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 19.5 min)

5-(4-chlorophenyl)-3-(2-isocyanoethyl)-1,3,4-oxadiazol-2(3H)-one (5)

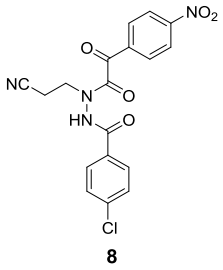


5

R_f = 0.43 (EtOAc : hexane = 1 : 2), R_f = 0.50 (EtOAc : hexane : CH_2Cl_2 = 1 : 2 : 2); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 8.7 Hz, 2H, aryl-CH), 7.44 (d, *J* = 8.22 Hz, 2H, aryl-CH), 4.09 (t, 2H), 2.88 (t, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 153.3, 152.7, 138.3, 129.5 (2C), 127.1 (2C), 121.8, 116.1, 41.6, 17.1; ESI-MS *m/z* 250.00 $(M+H)^+$; HR-MS (FAB+) *m/z* 250.0381 (calculated for $C_{11}H_9O_2N_3Cl^+$ $[M+H]^+$ 250.0383); FT-IR (KBr) 3725, 2349, 2309, 1769, 1490, 1457, 1406, 1362, 1092, 1011, 838, 742, 679, 656 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 17.7 min)

8. [Part 2] Synthesis of 8

4-chloro-N'-(2-cyanoethyl)-N'-(2-(4-nitrophenyl)-2-oxoacetyl)benzohydrazide (8)



8

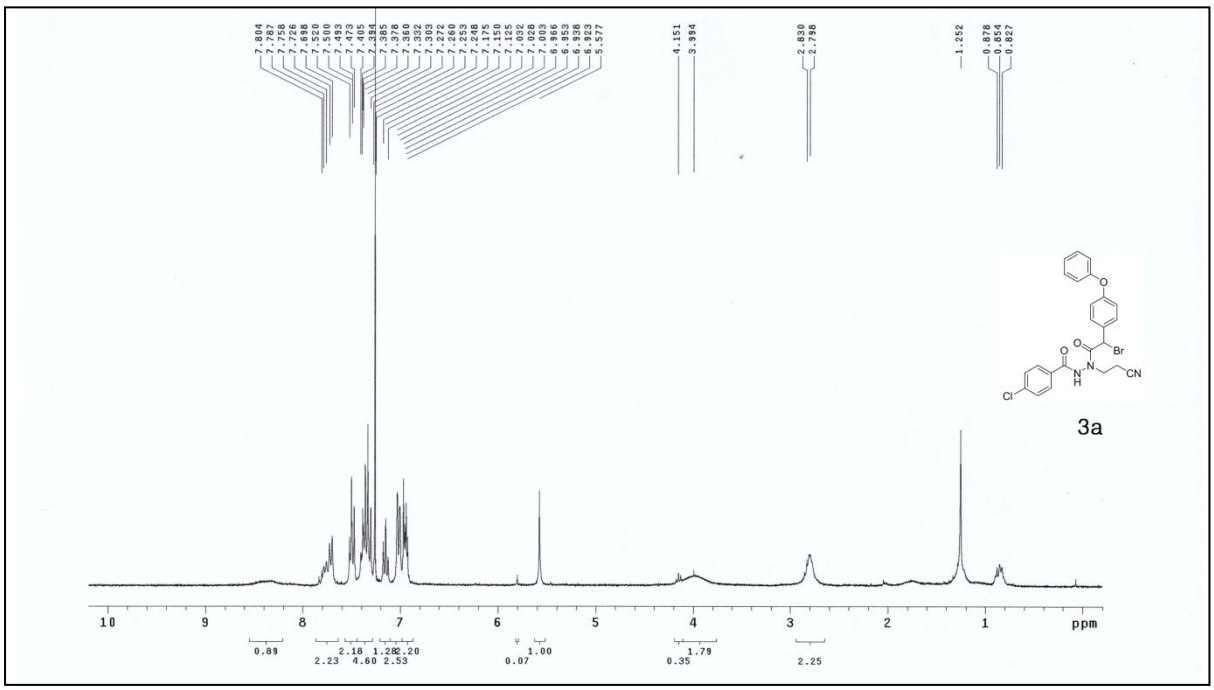
A 10 mL round-bottom flask equipped with a stir bar was charged with compound **4i** (12 mg, 0.031 mmol, 1.00 eq.), anhydrous acetonitrile (4 mL) and K_2CO_3 (10.0 eq.). The reaction mixture was stirred at room temperature for 3h and was observed by TLC. The reaction mixture was filtered to remove K_2CO_3 and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (EtOAc : CH_2Cl_2 :hexane = 1 : 1 : 2) to afford **8** (4 mg, 36% yield) as white solid

R_f = 0.23 (EtOAc : CH_2Cl_2 :hexane = 1 : 1 : 2); ^{1}H NMR (800 MHz, DMSO) δ 11.5 (s, 1H, amide-NH), 8.37 (d, 2H, *J* = 8.8 Hz, aryl-CH), 8.14 (d, 2H, *J* = 8.88 Hz, aryl-CH), 7.61 (d, 2H, *J* = 8.64 Hz, aryl-CH), 7.54 (d, 2H, *J* = 8.64 Hz, aryl-CH), 4.28 (br, 1H), 3.74 (br, 1H), 2.92 (br, 2H),; ^{13}C-NMR (200 MHz, DMSO) δ 167.0, 165.6, 150.9, 137.5, 136.0, 130.7 (2C), 129.7, 129.4 (2C), 128.7 (2C), 124.1 (2C), 119.0, 42.8, 15.7; ESI-MS *m/z* 401.02 $(M+H)^+$; HR-MS (FAB+) *m/z* 401.0665 (calculated for

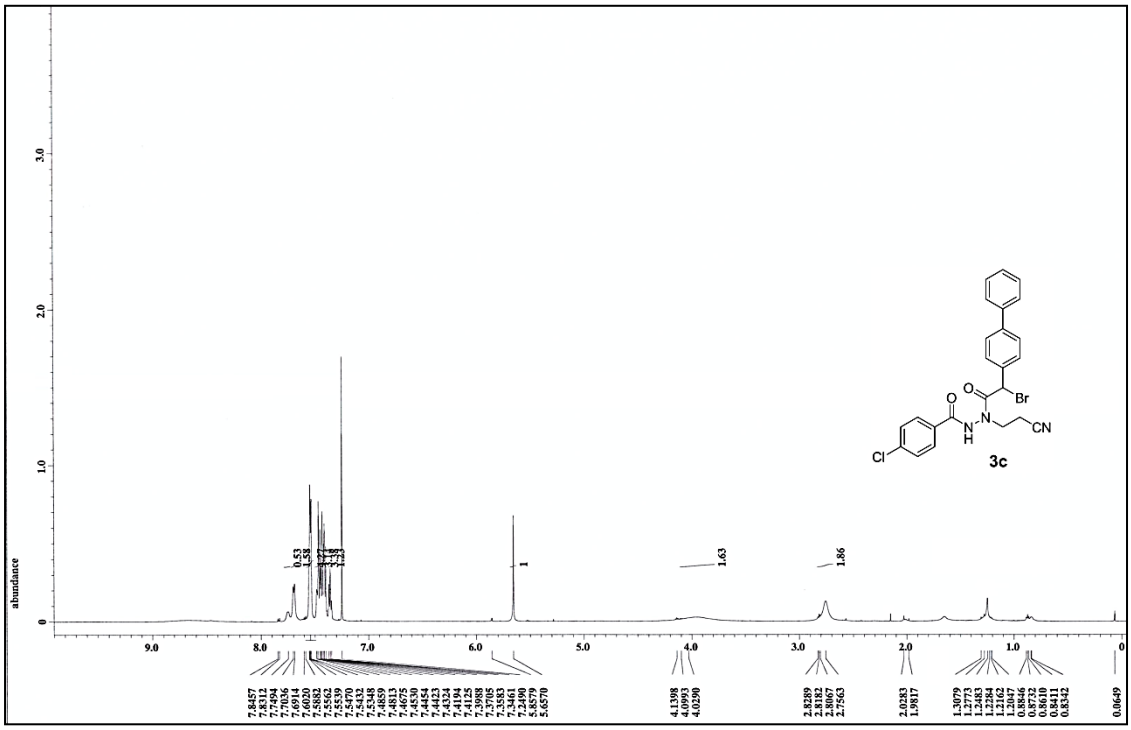
$C_{18}H_{14}O_5N_4Cl^+$ $[M+H]^+$ 401.0653); FT-IR (KBr) 3282, 2926, 2253, 1785, 1689, 1598, 1527, 1485, 1407, 1349, 1271, 1227, 1196, 1095, 1014, 848, 788, 759, 725, 703, 679 cm^{-1}; HPLC flow rate = 1 mL/min, relative purity 100%, (t = 20.5 min)

9. [Part 2] NMR Spectra

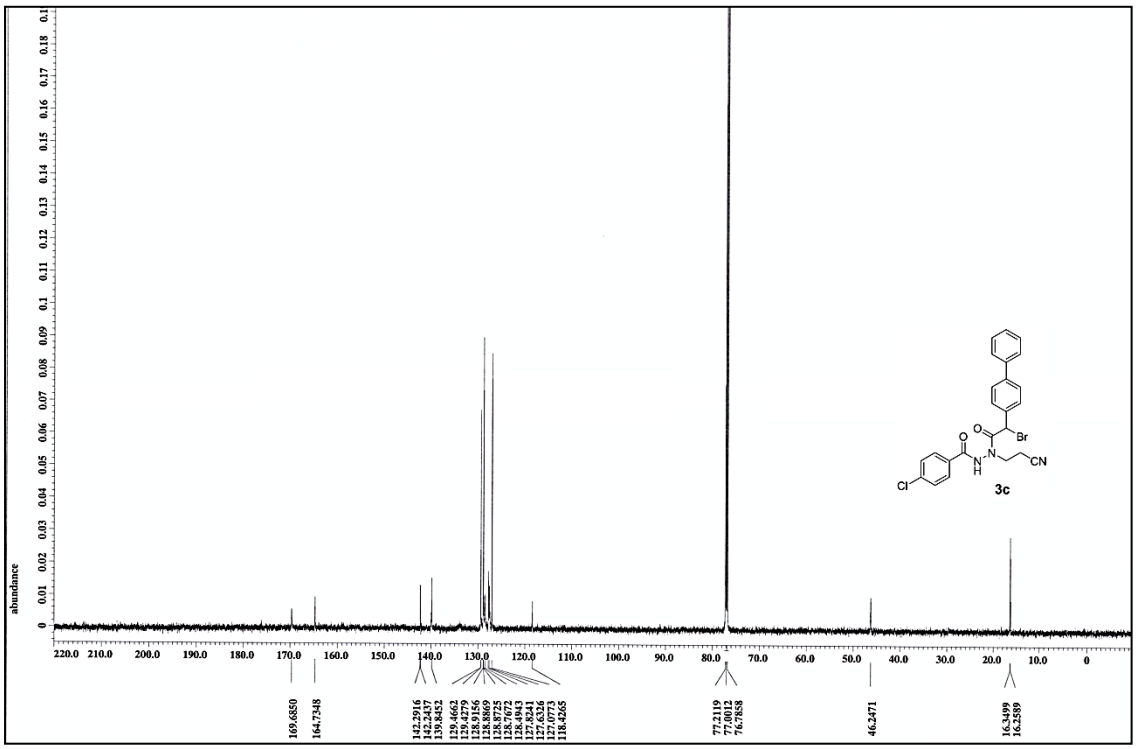
^{1}H NMR of compound **3a** (300 MHz, $CDCl_3$)



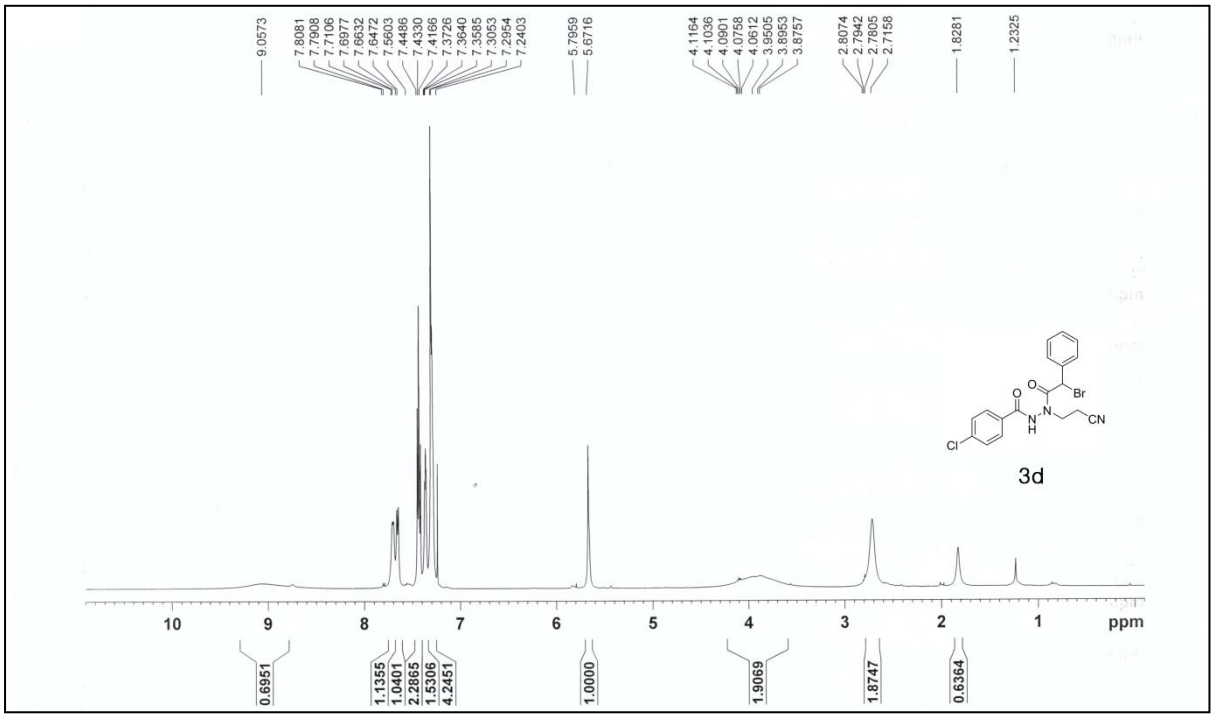
^{1}H NMR of compound **3c** (600 MHz, $CDCl_3$)



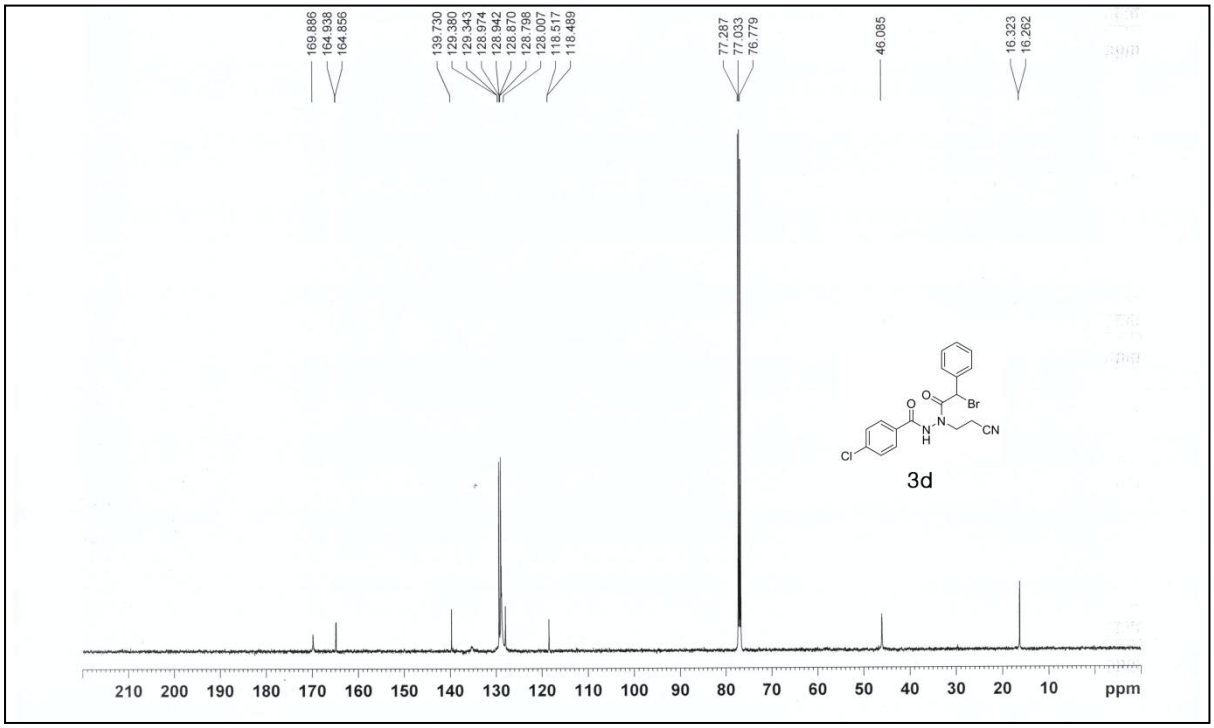
^{13}C NMR of compound **3c** (150 MHz, $CDCl_3$)



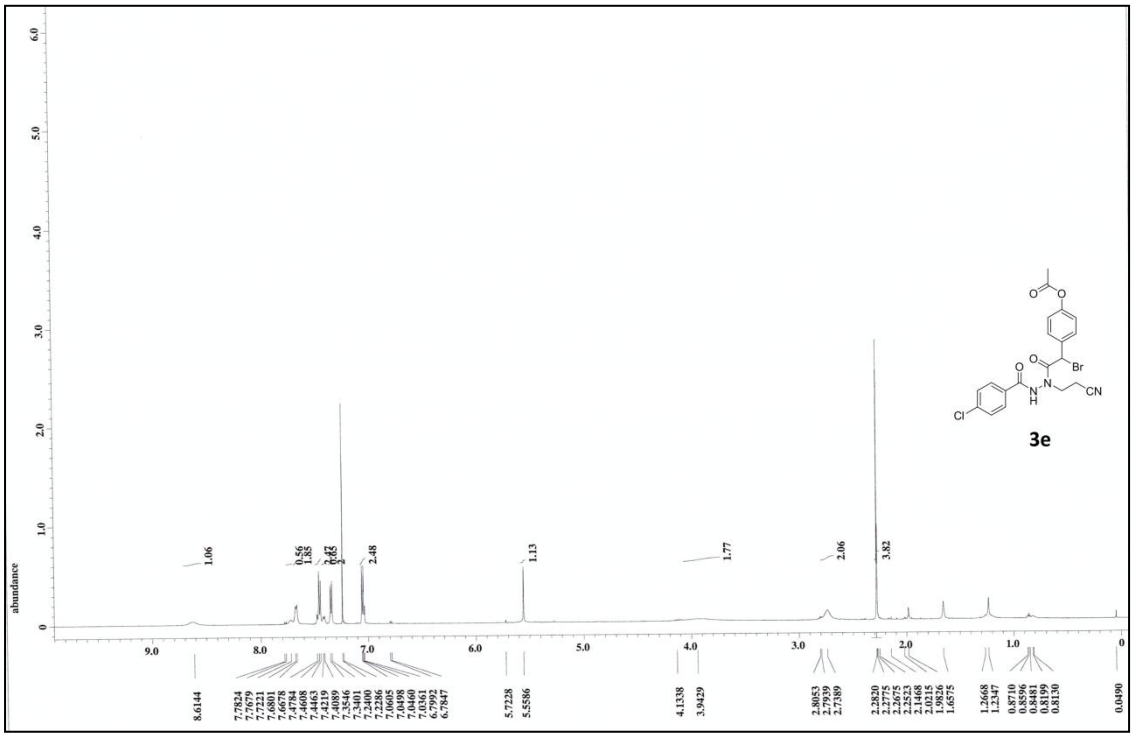
^{1}H NMR of compound **3d** (500 MHz, $CDCl_3$)



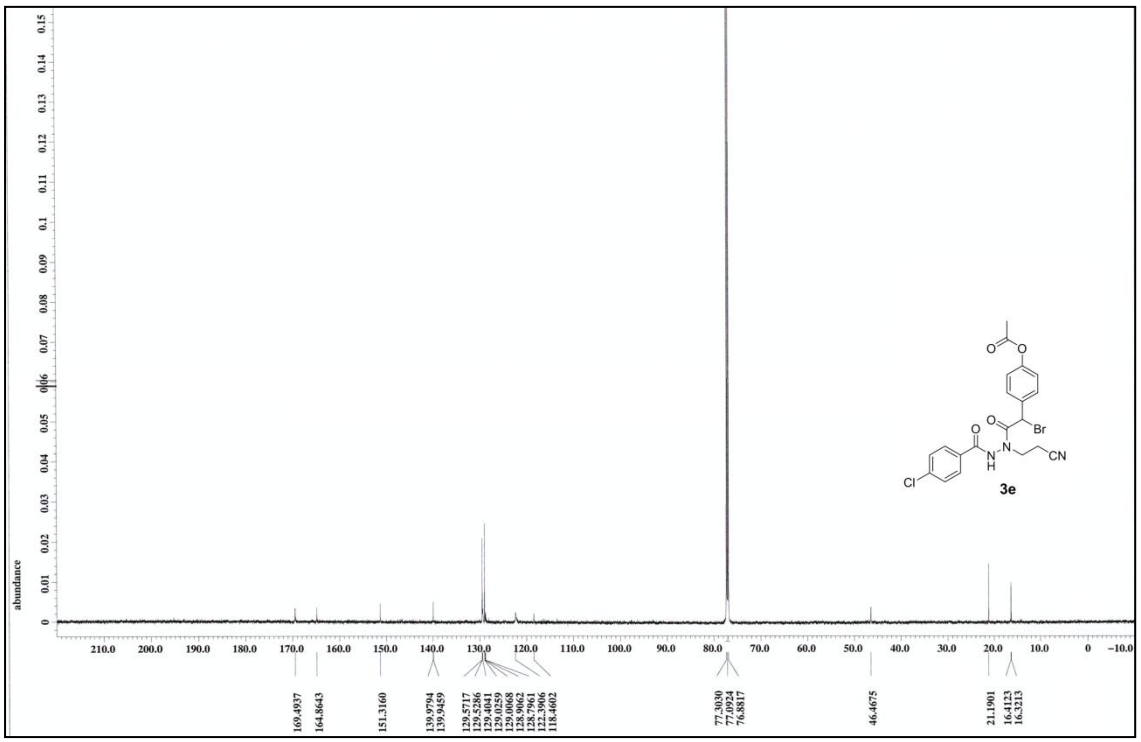
^{13}C NMR of compound **3d** (125 MHz, $CDCl_3$)



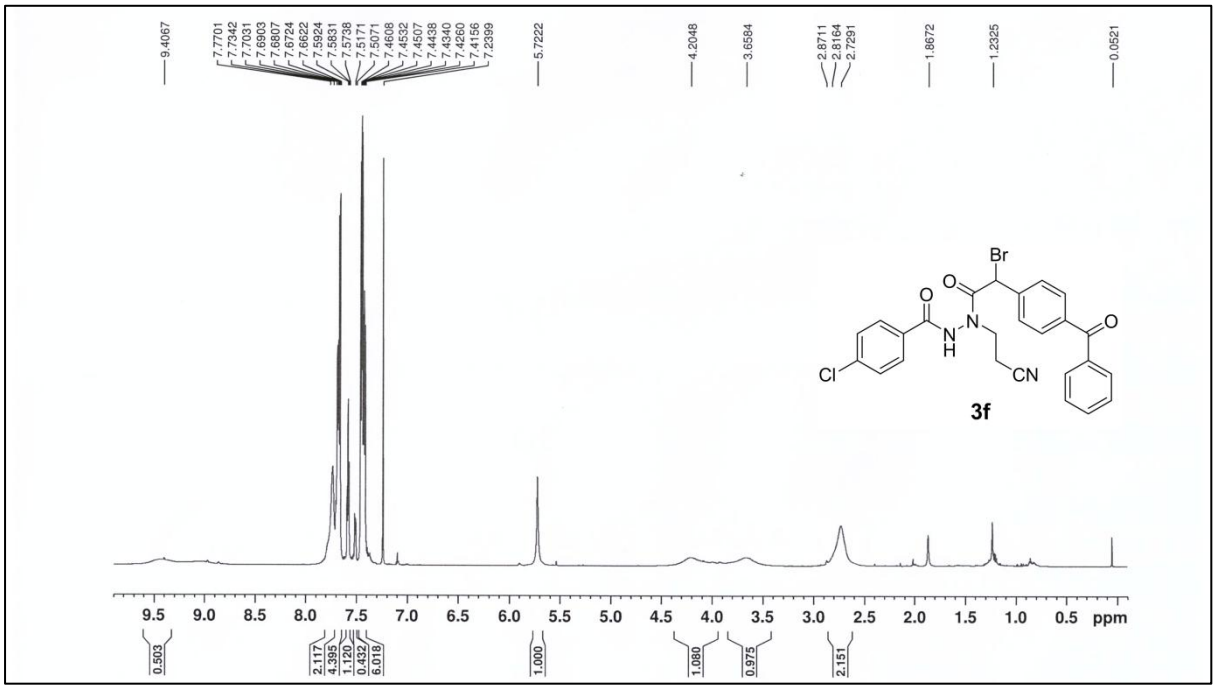
^{1}H NMR of compound **3e** (600 MHz, $CDCl_3$)



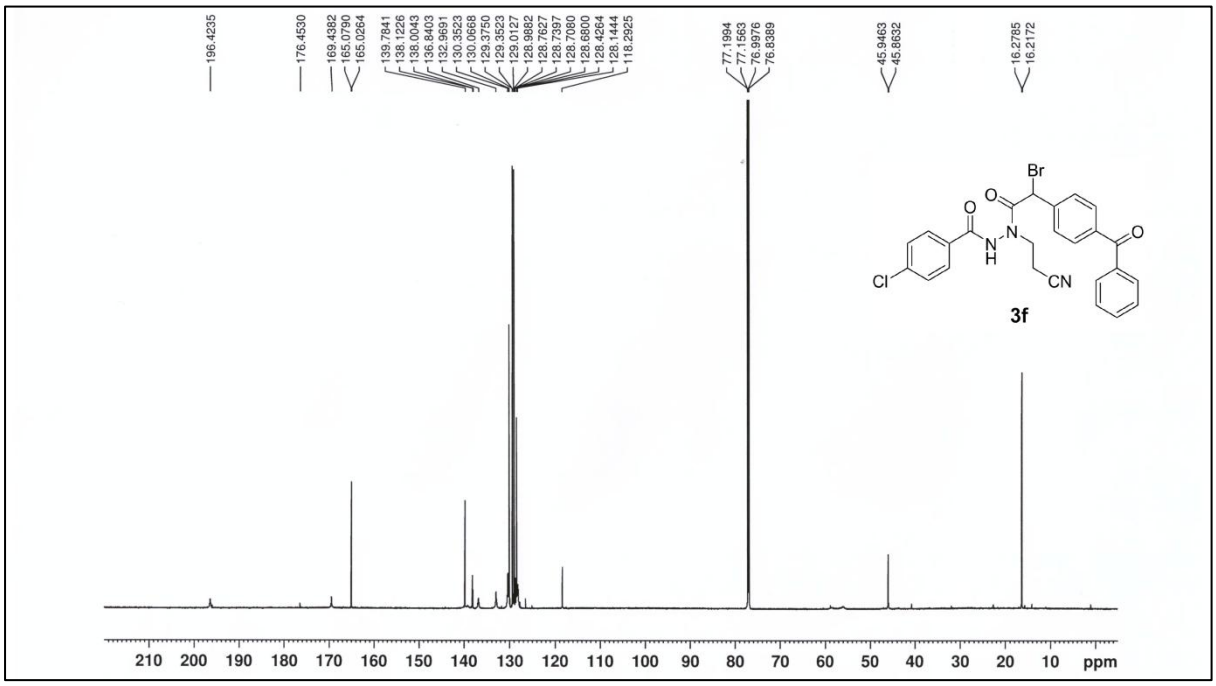
^{13}C NMR of compound **3e** (150 MHz, $CDCl_3$)



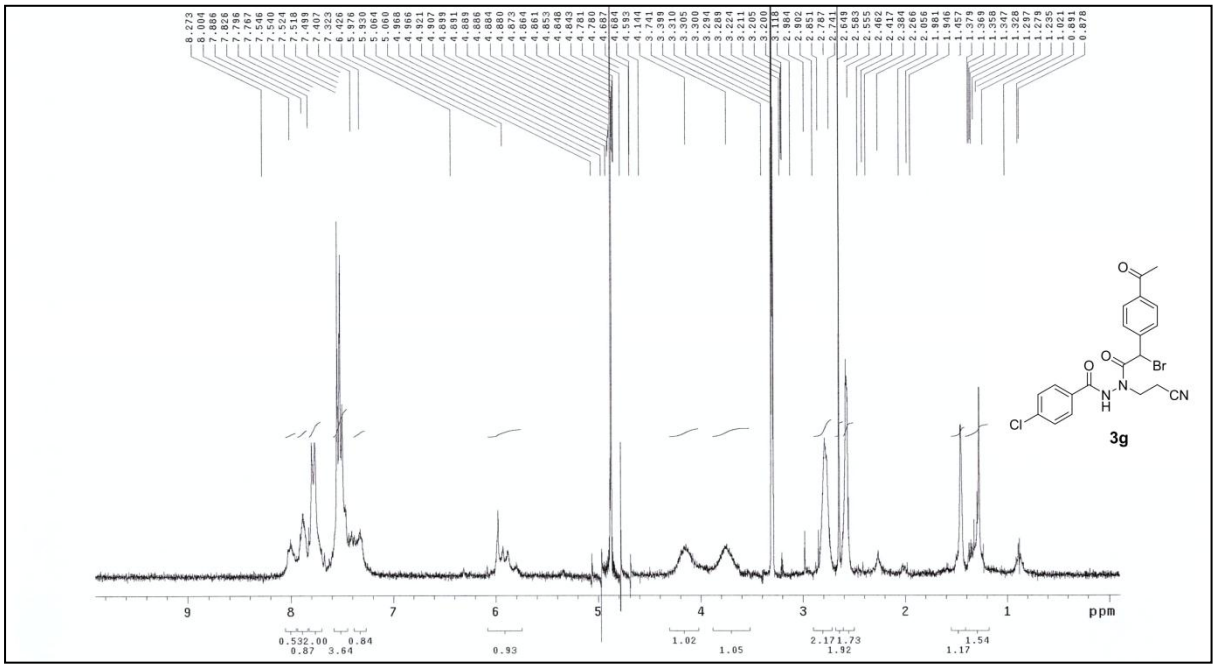
^{1}H NMR of compound **3f** (800 MHz, $CDCl_3$)



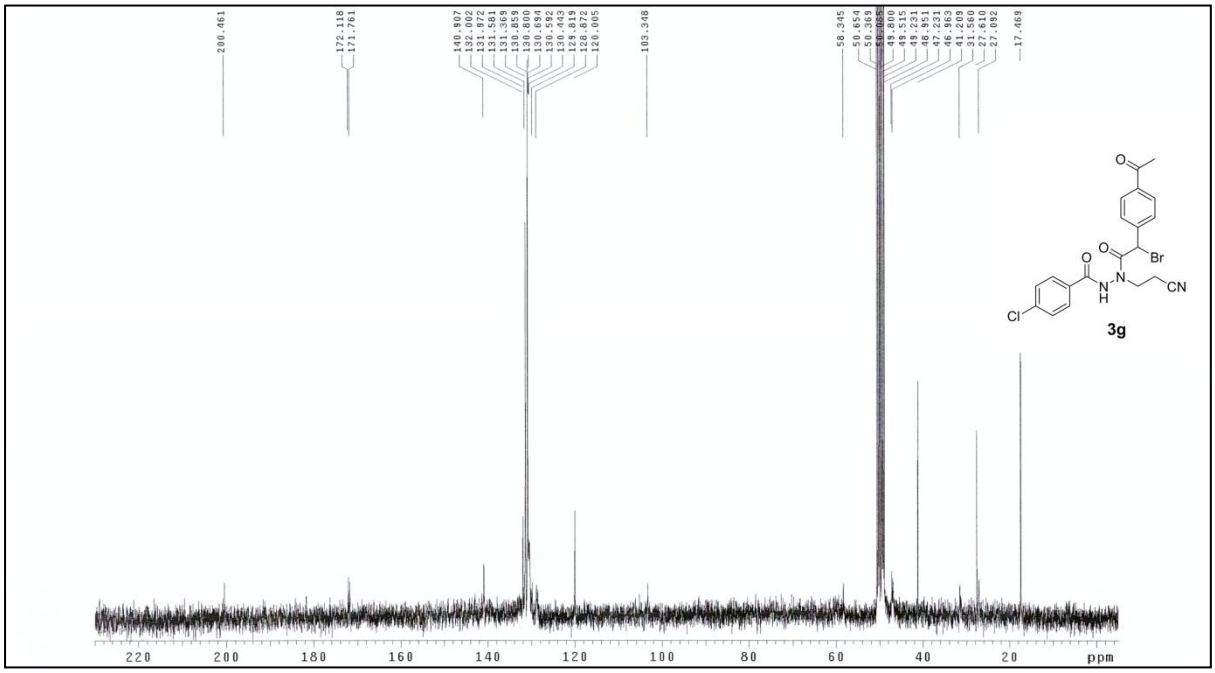
^{13}C NMR of compound **3f** (200 MHz, $CDCl_3$)



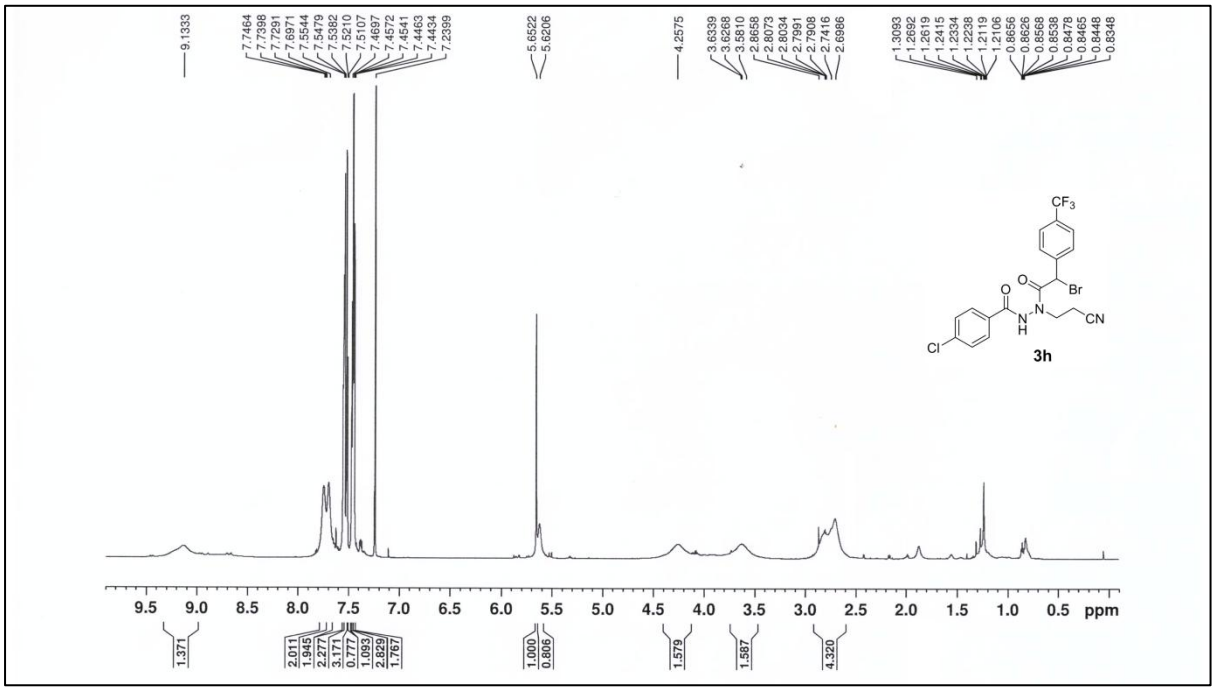
^{1}H NMR of compound **3g** (300 MHz, CD_3OD)



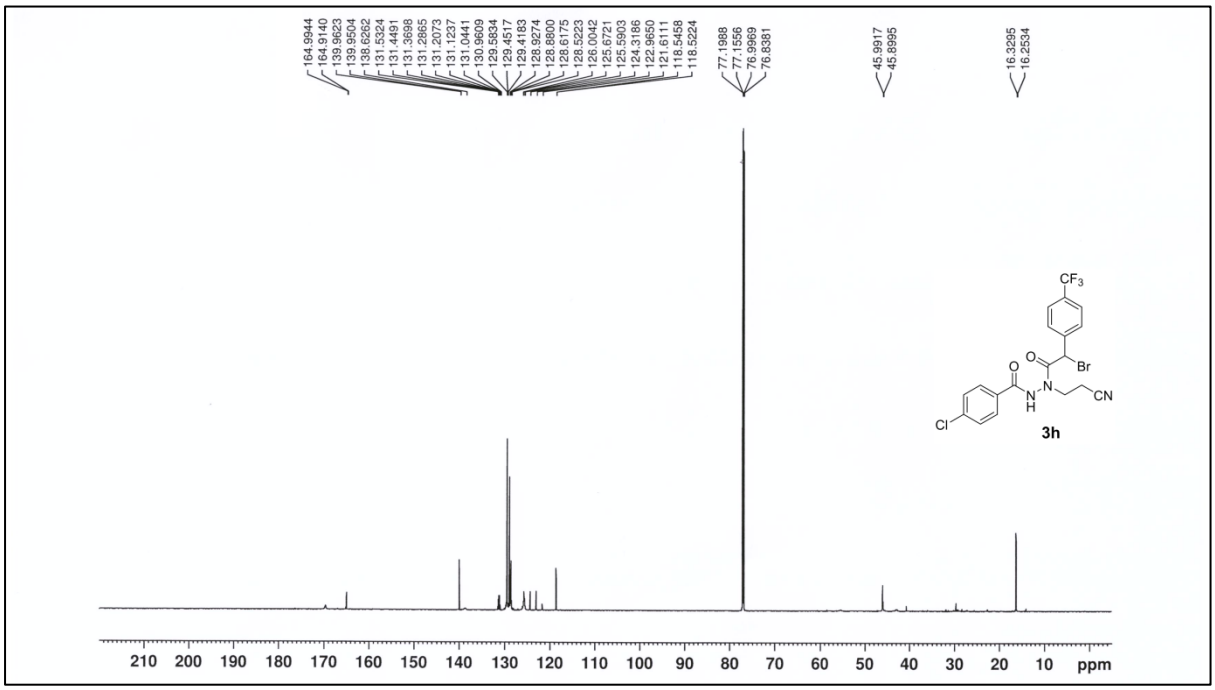
^{13}C NMR of compound **3g** (75 MHz, CD_3OD)



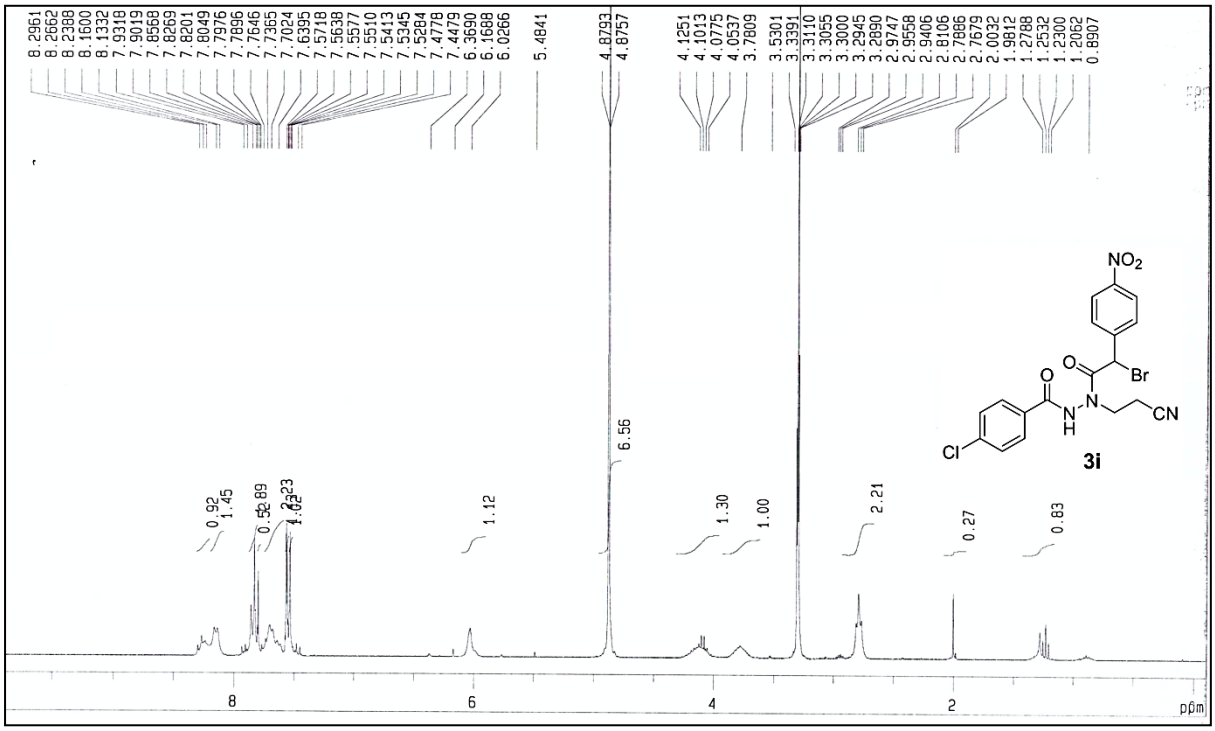
^{1}H NMR of compound **3h** (800 MHz, $CDCl_3$)



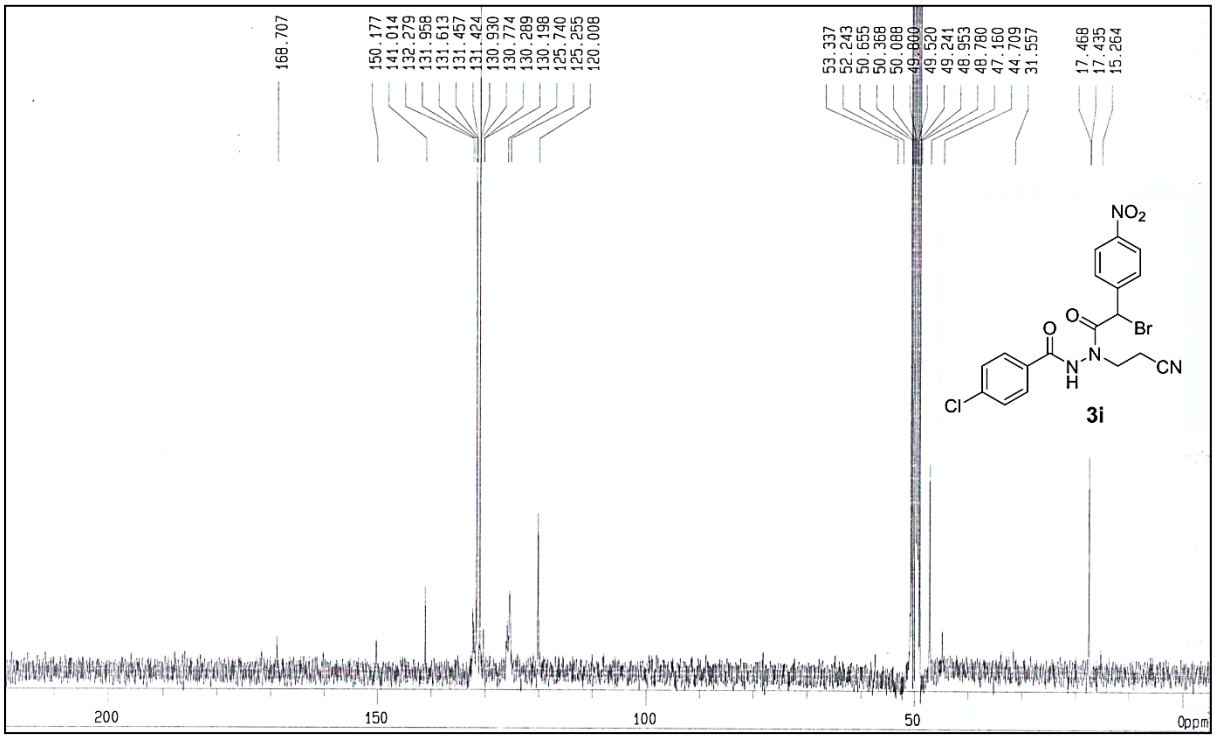
^{13}C NMR of compound **3h** (200 MHz, $CDCl_3$)



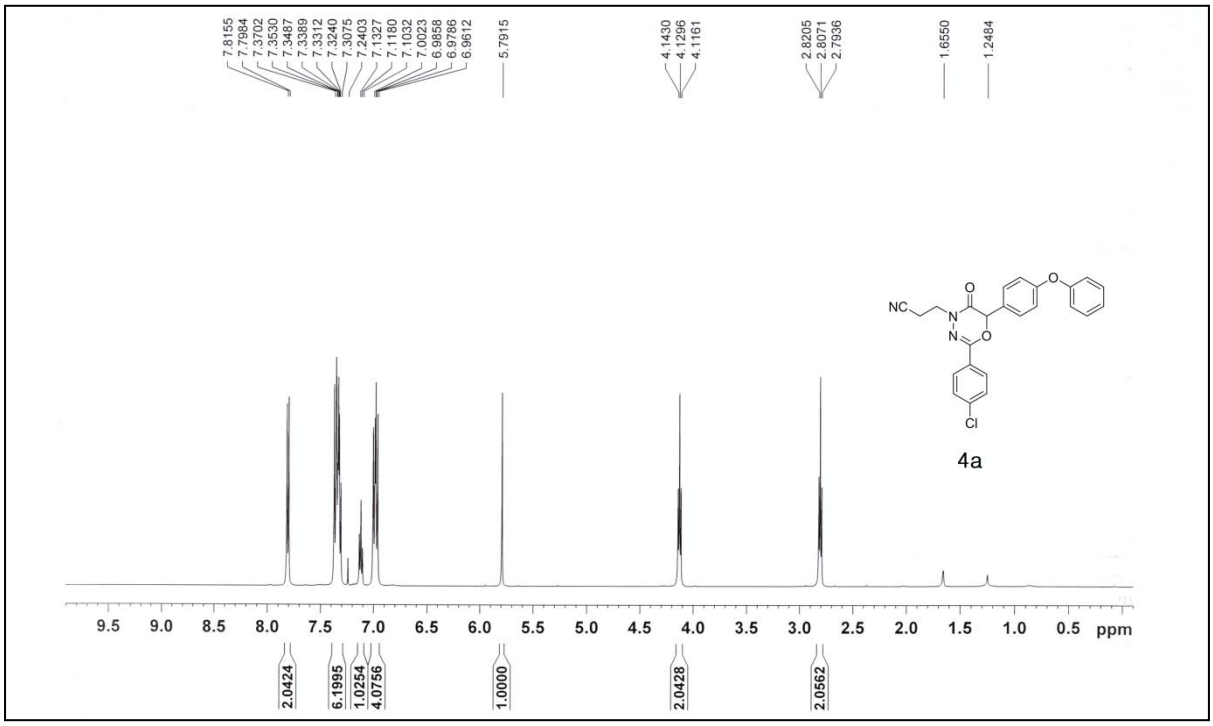
^{1}H NMR of compound **3i** (300 MHz, CD_3OD)



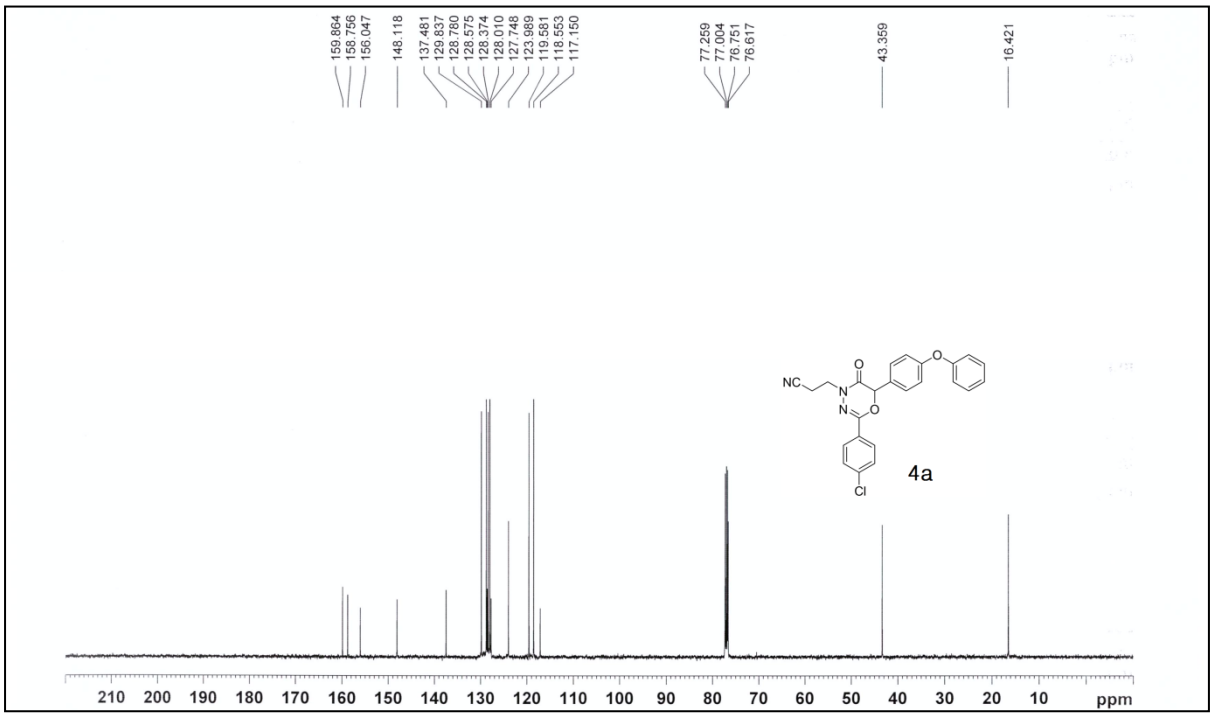
^{13}C NMR of compound **3i** (75 MHz, CD_3OD)



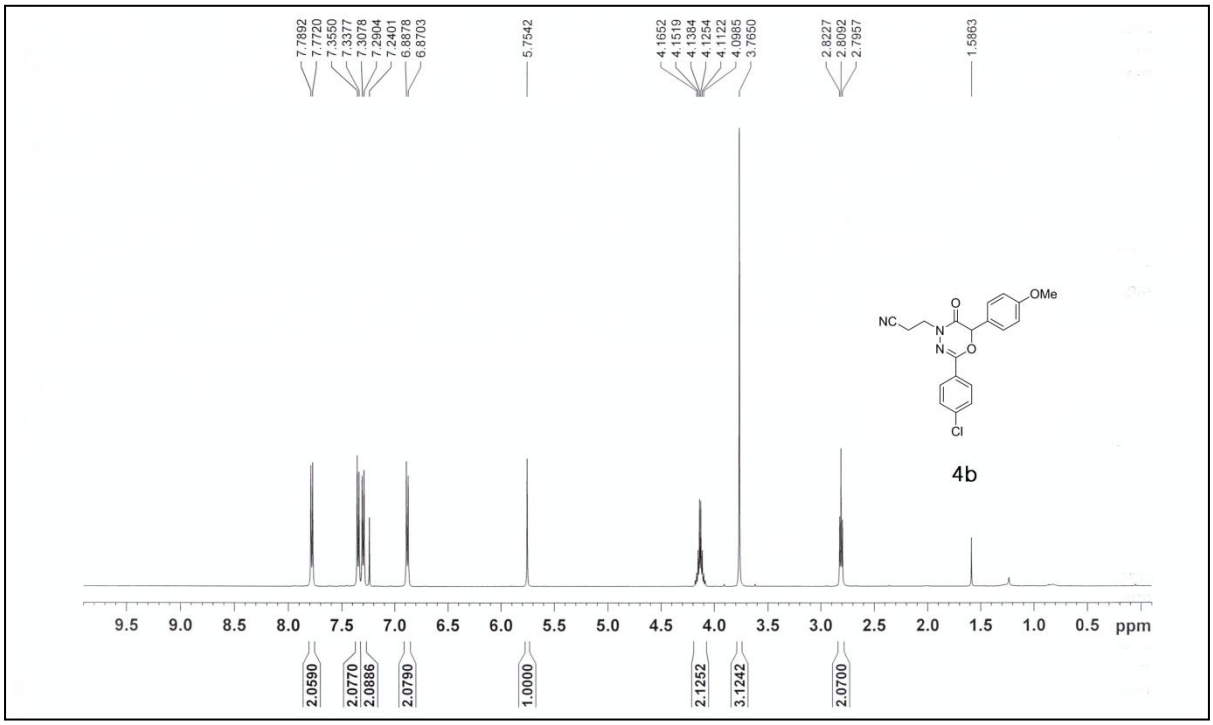
^{1}H NMR of compound **4a** (500 MHz, $CDCl_3$)



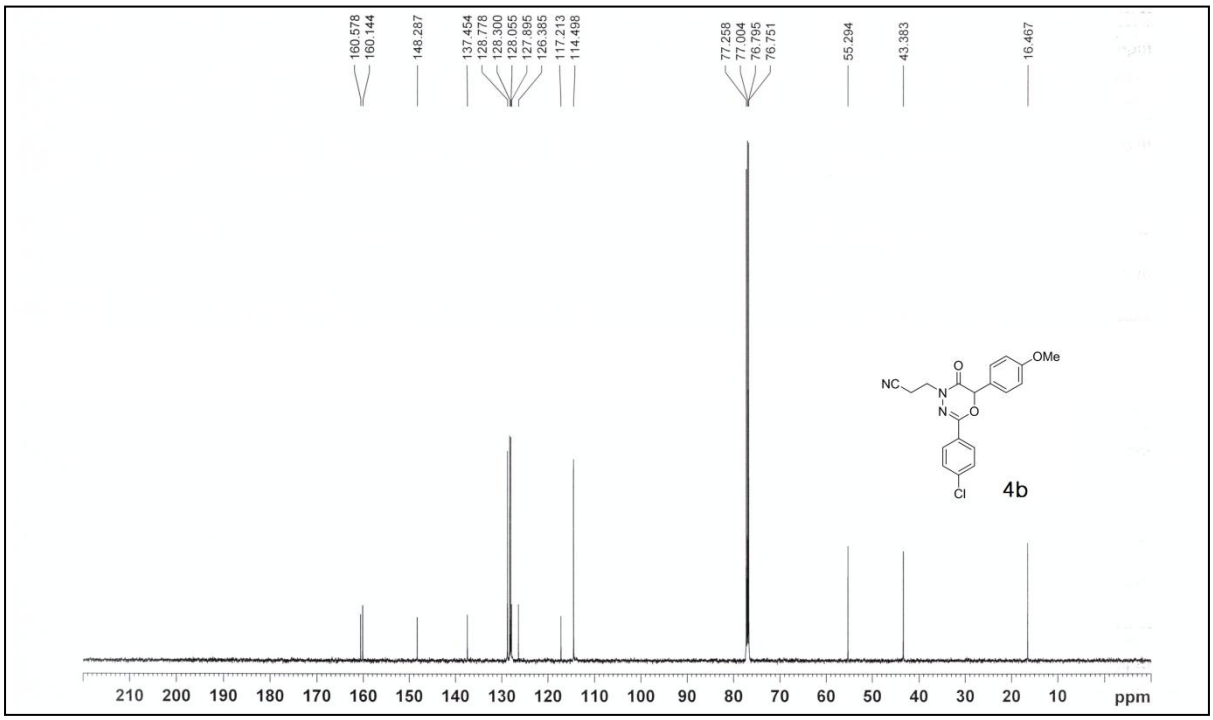
^{13}C NMR of compound **4a** (125 MHz, $CDCl_3$)



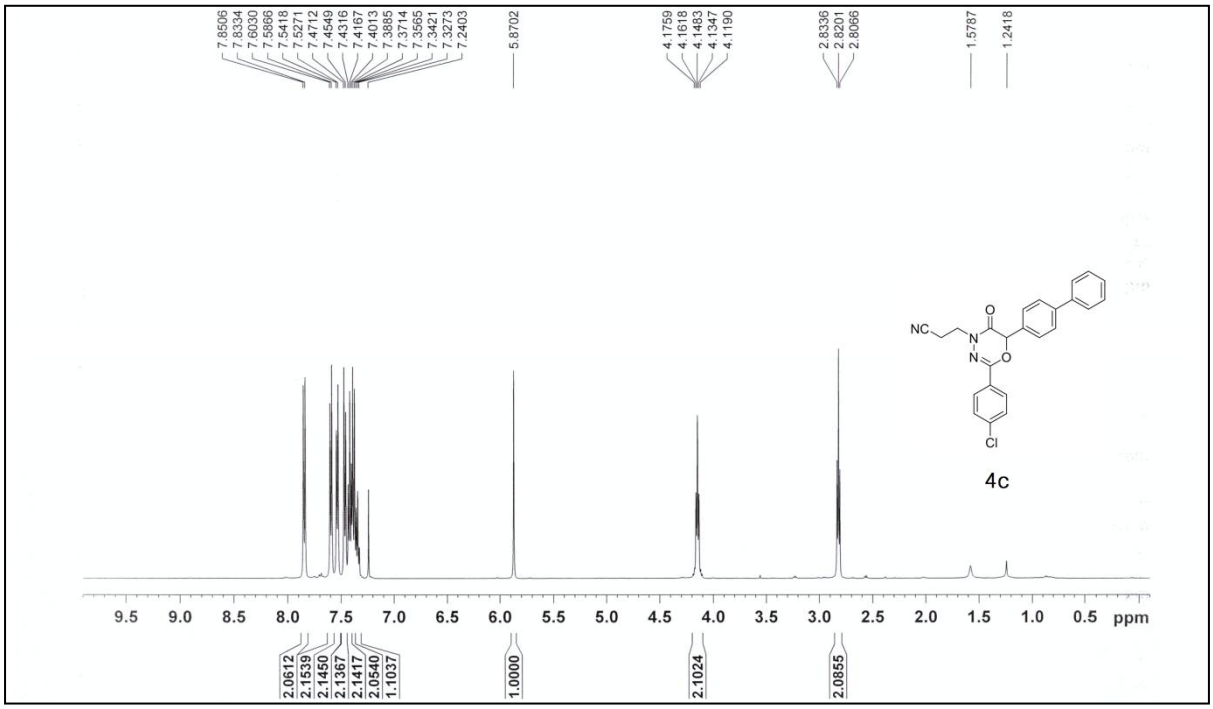
^{1}H NMR of compound **4b** (500 MHz, $CDCl_3$)



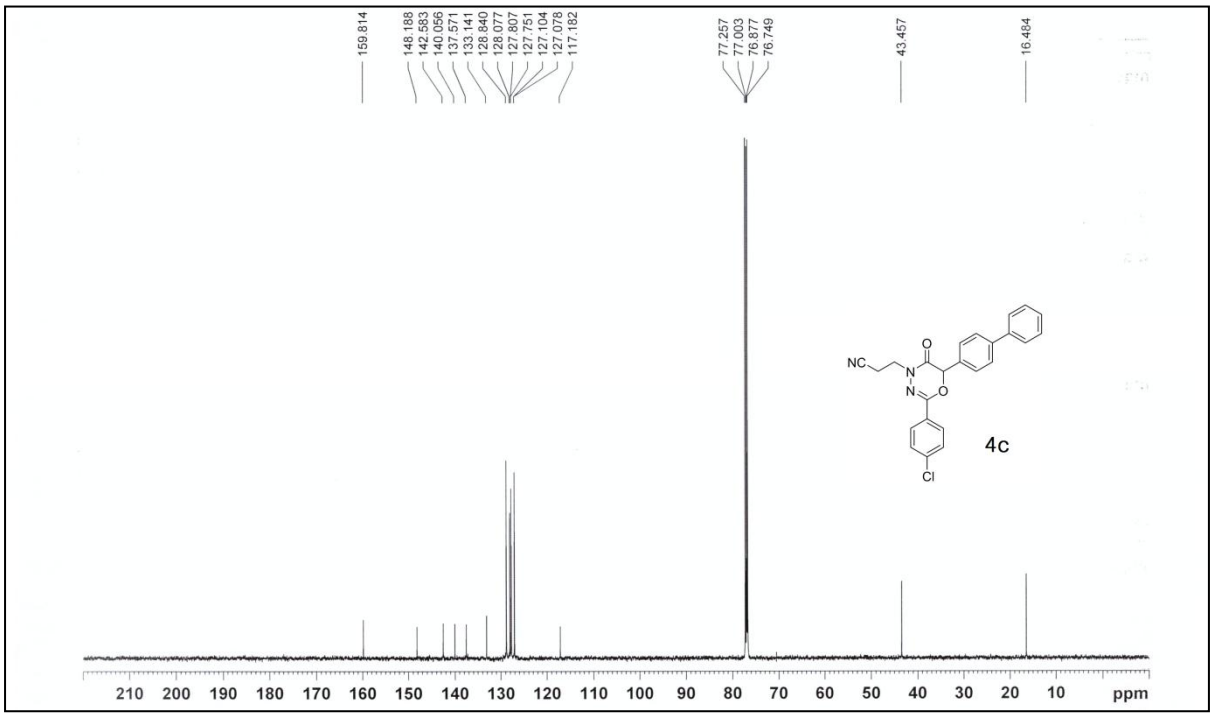
^{13}C NMR of compound **4b** (125 MHz, $CDCl_3$)



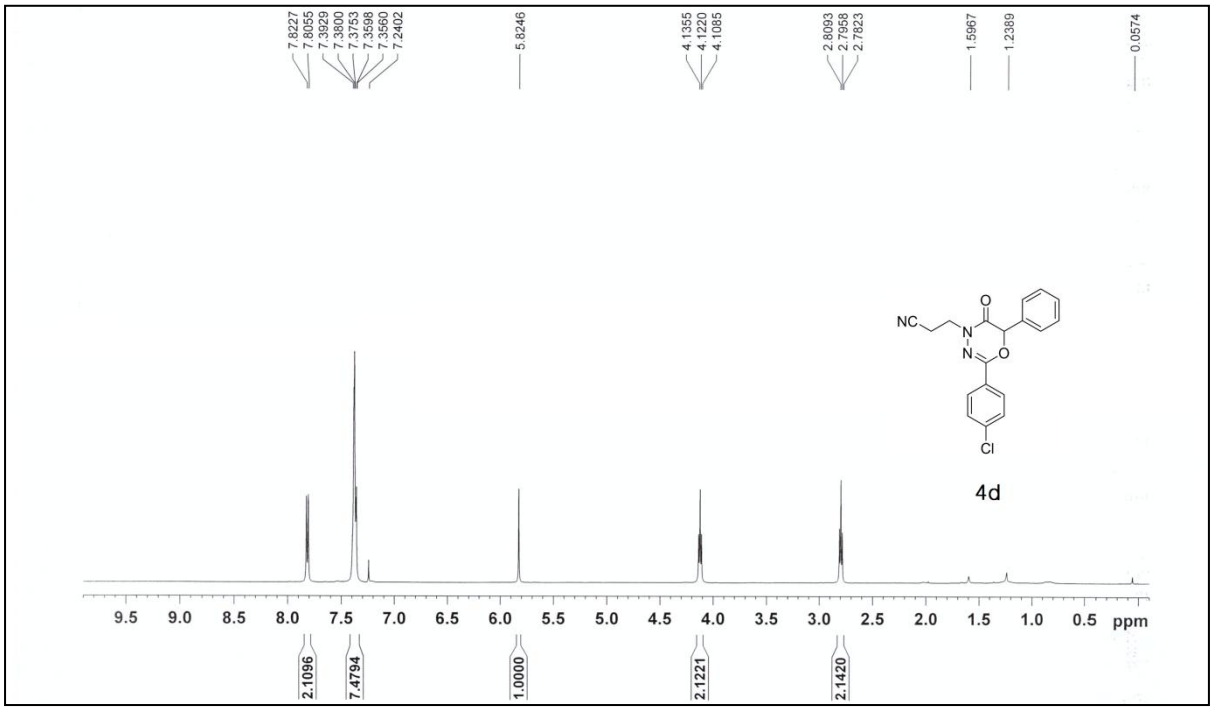
^{1}H NMR of compound **4c** (500 MHz, $CDCl_3$)



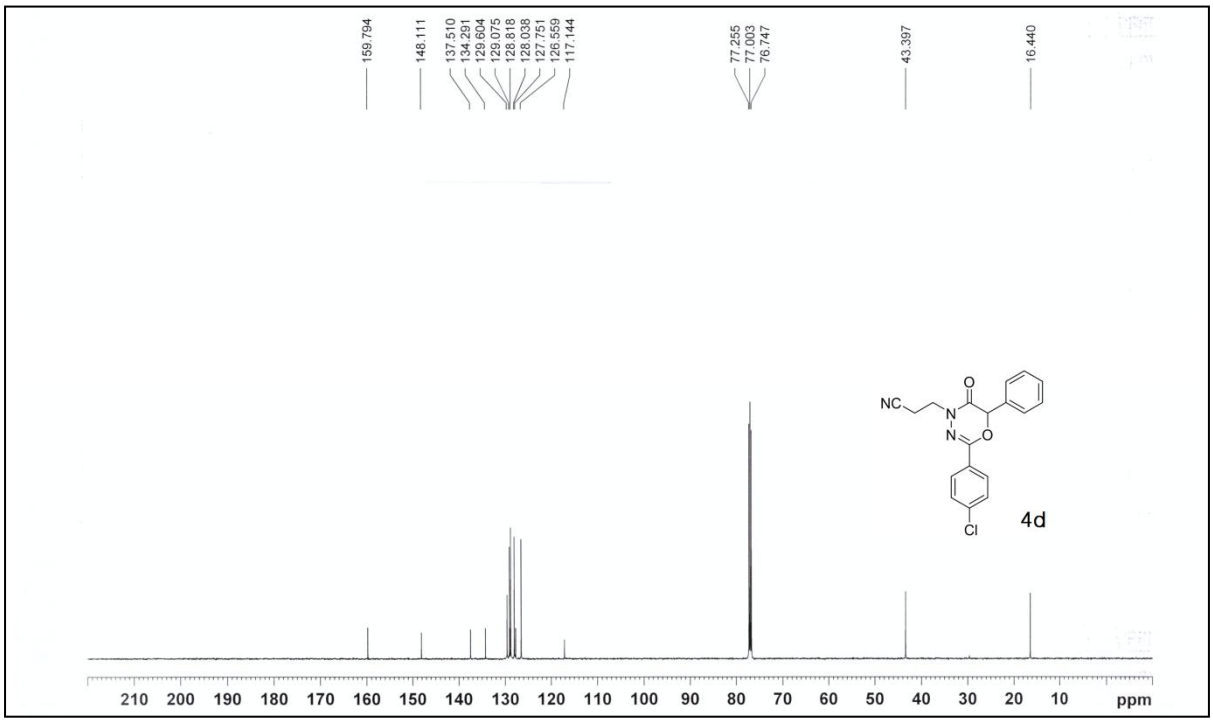
^{13}C NMR of compound **4c** (125 MHz, $CDCl_3$)



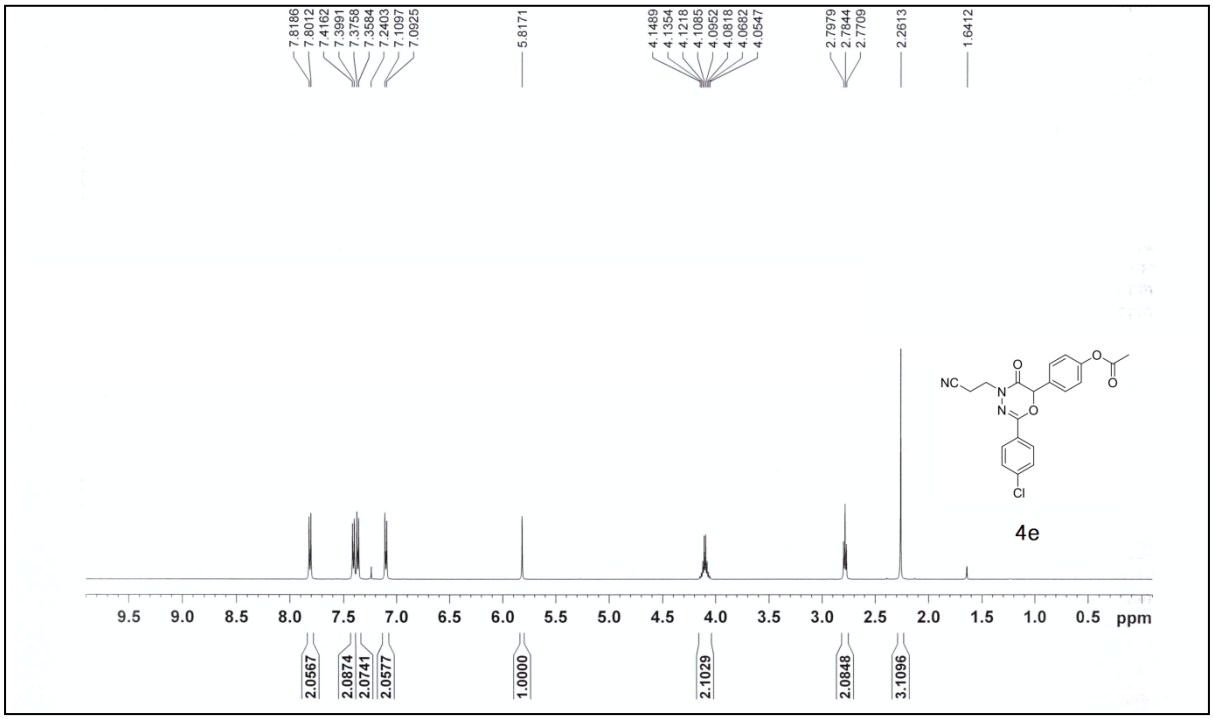
^{1}H NMR of compound **4d** (500 MHz, $CDCl_3$)



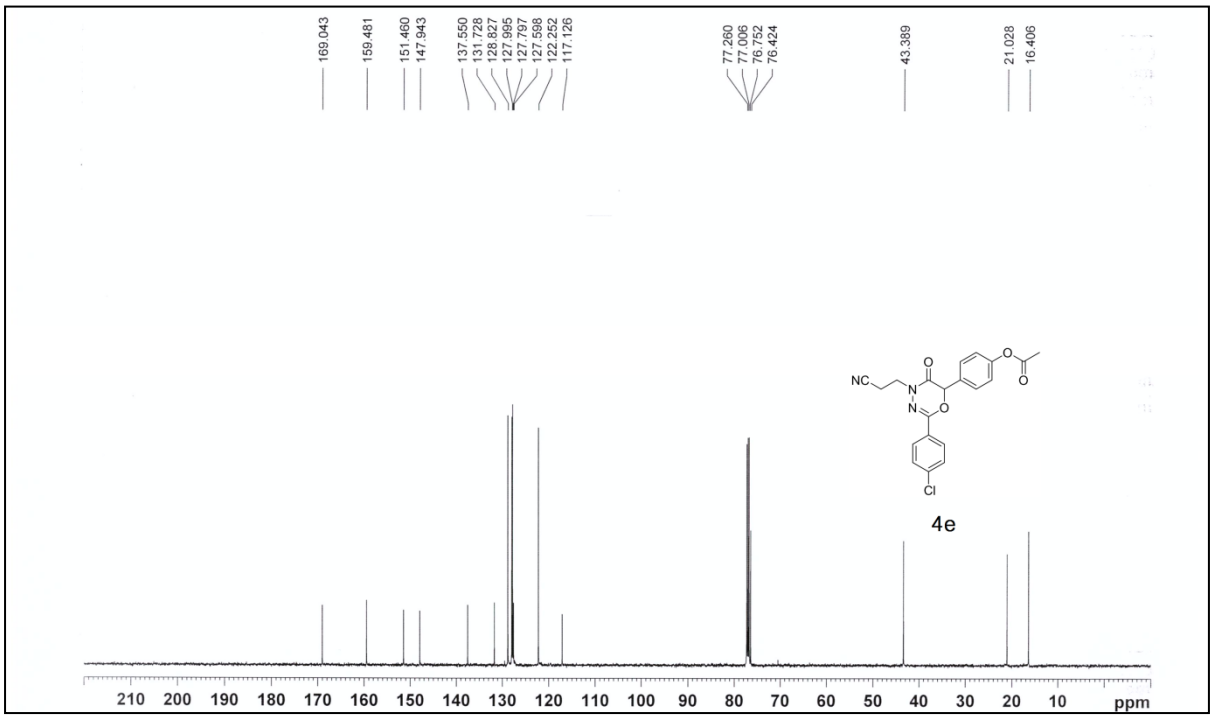
^{13}C NMR of compound **4d** (125 MHz, $CDCl_3$)



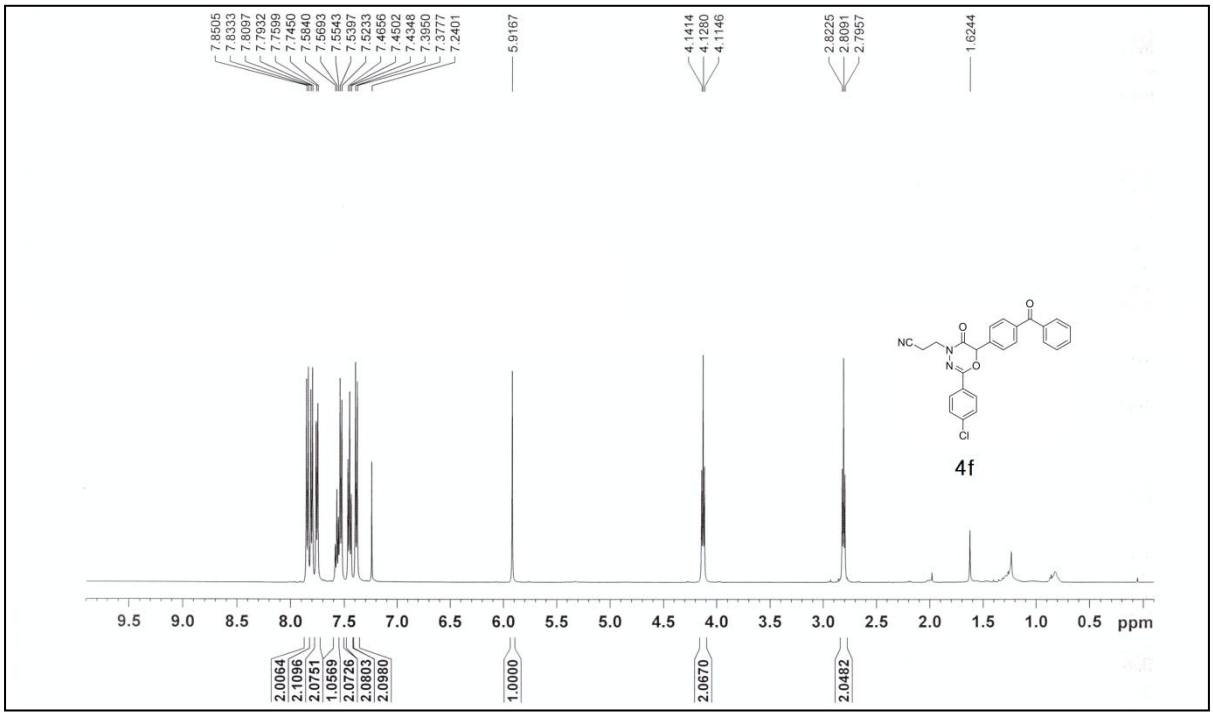
^{1}H NMR of compound **4e** (500 MHz, $CDCl_3$)



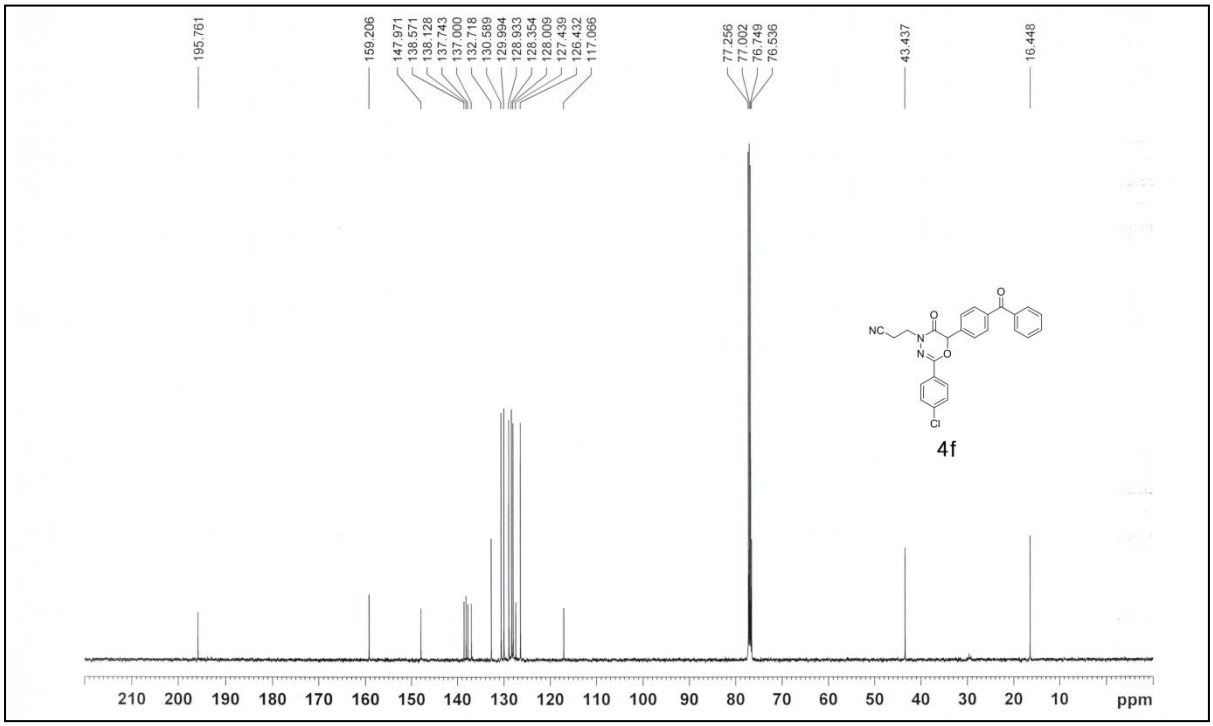
^{13}C NMR of compound **4e** (125 MHz, $CDCl_3$)



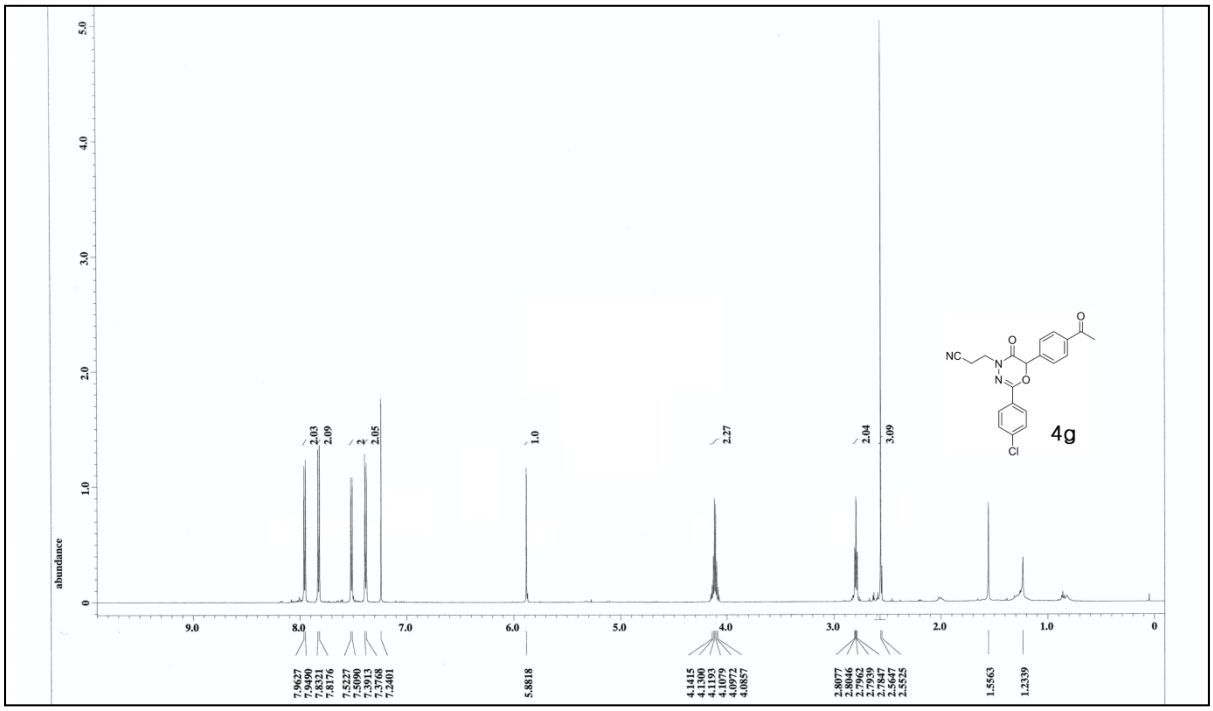
^{1}H NMR of compound **4f** (500 MHz, $CDCl_3$)



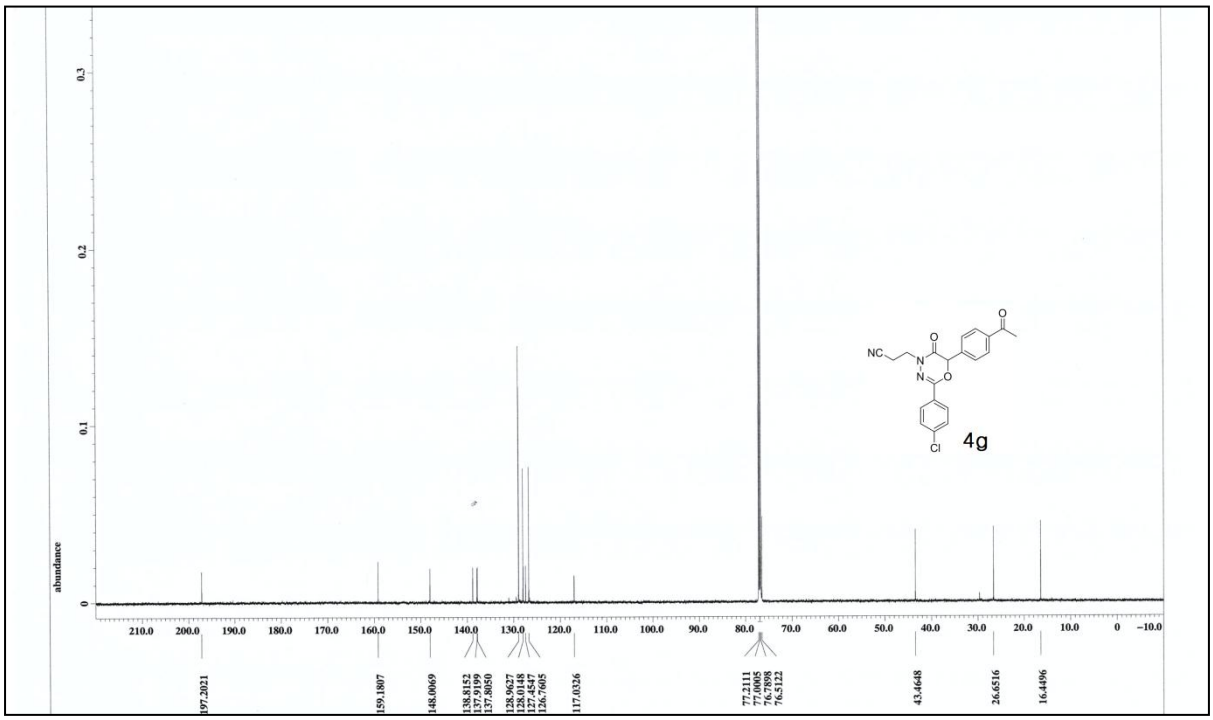
^{13}C NMR of compound **4f** (125 MHz, $CDCl_3$)



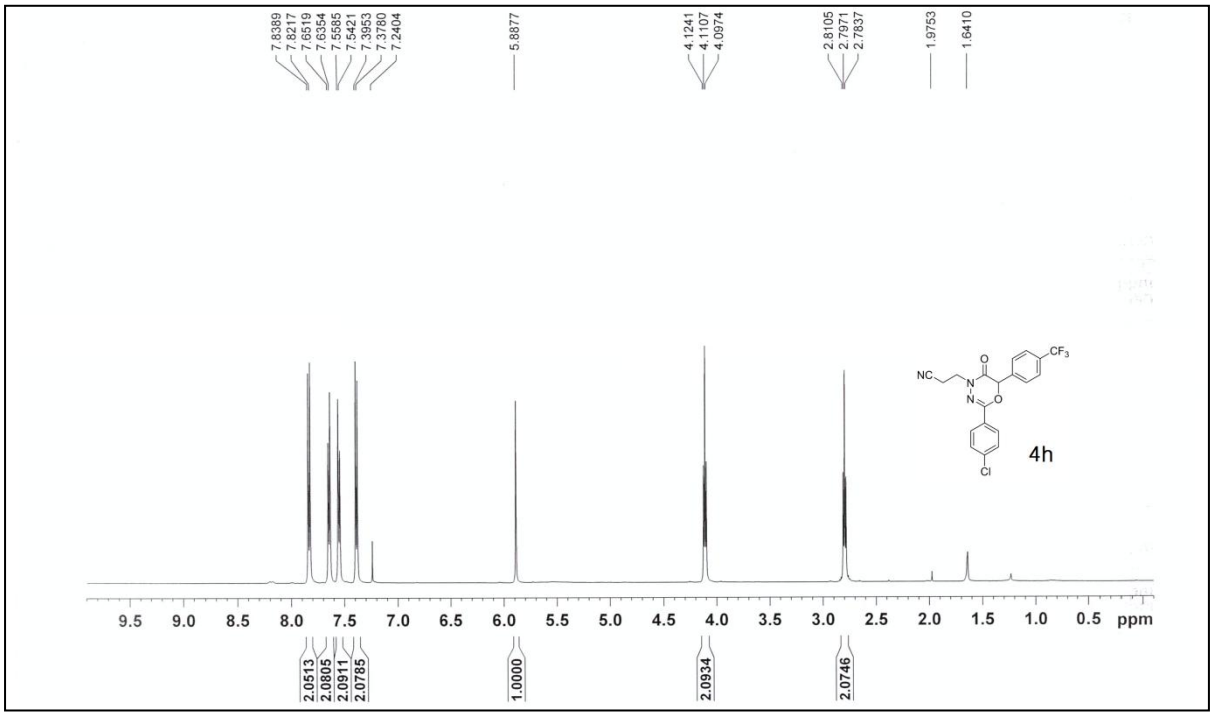
^{1}H NMR of compound **4g** (600 MHz, $CDCl_3$)



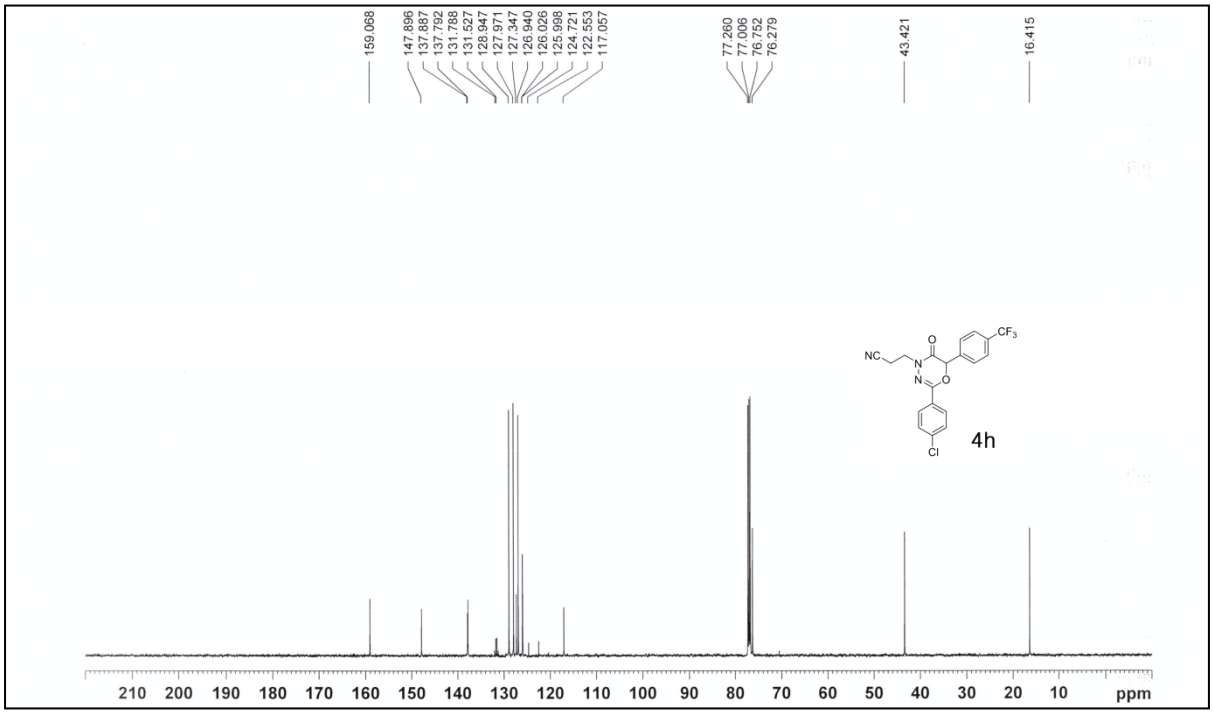
^{13}C NMR of compound **4g** (150 MHz, $CDCl_3$)



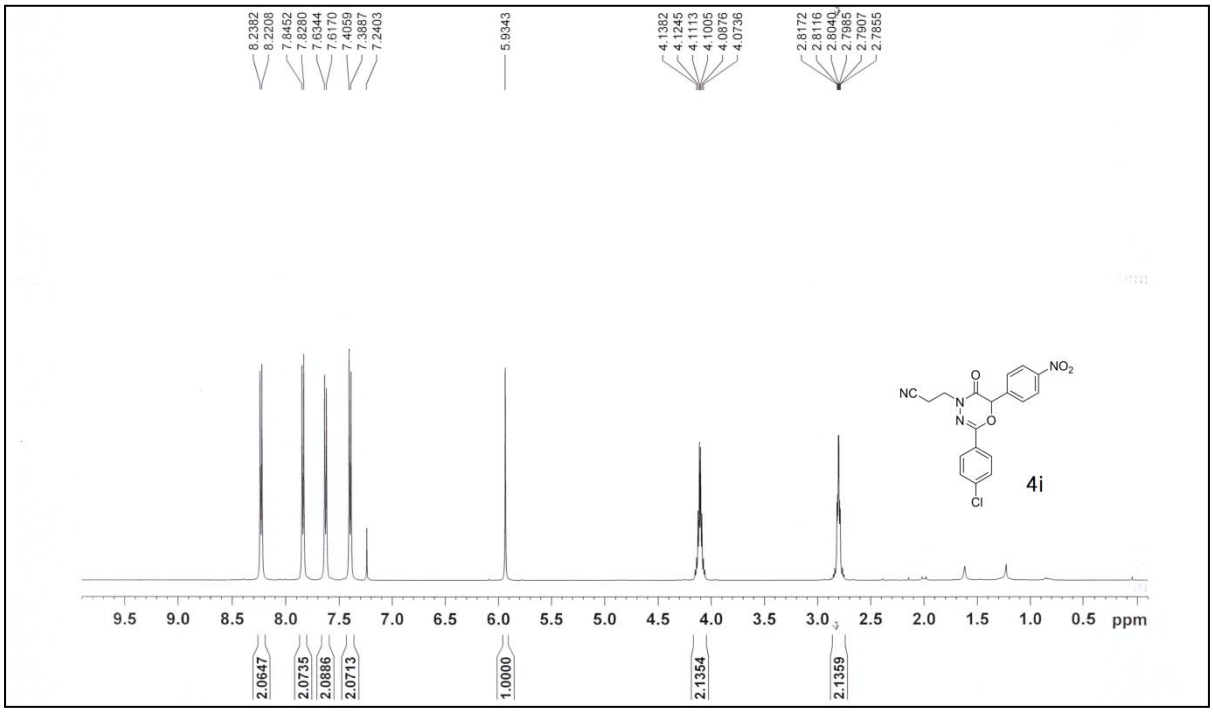
^{1}H NMR of compound **4h** (500 MHz, $CDCl_3$)



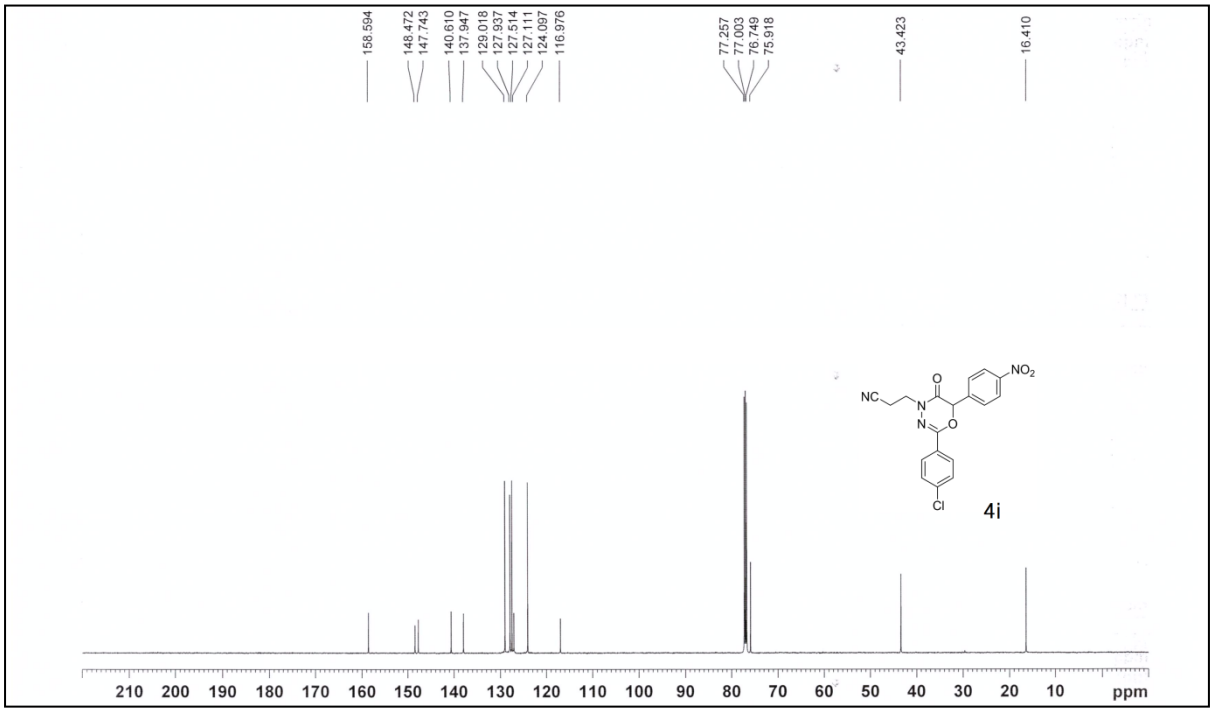
^{13}C NMR of compound **4h** (125 MHz, $CDCl_3$)



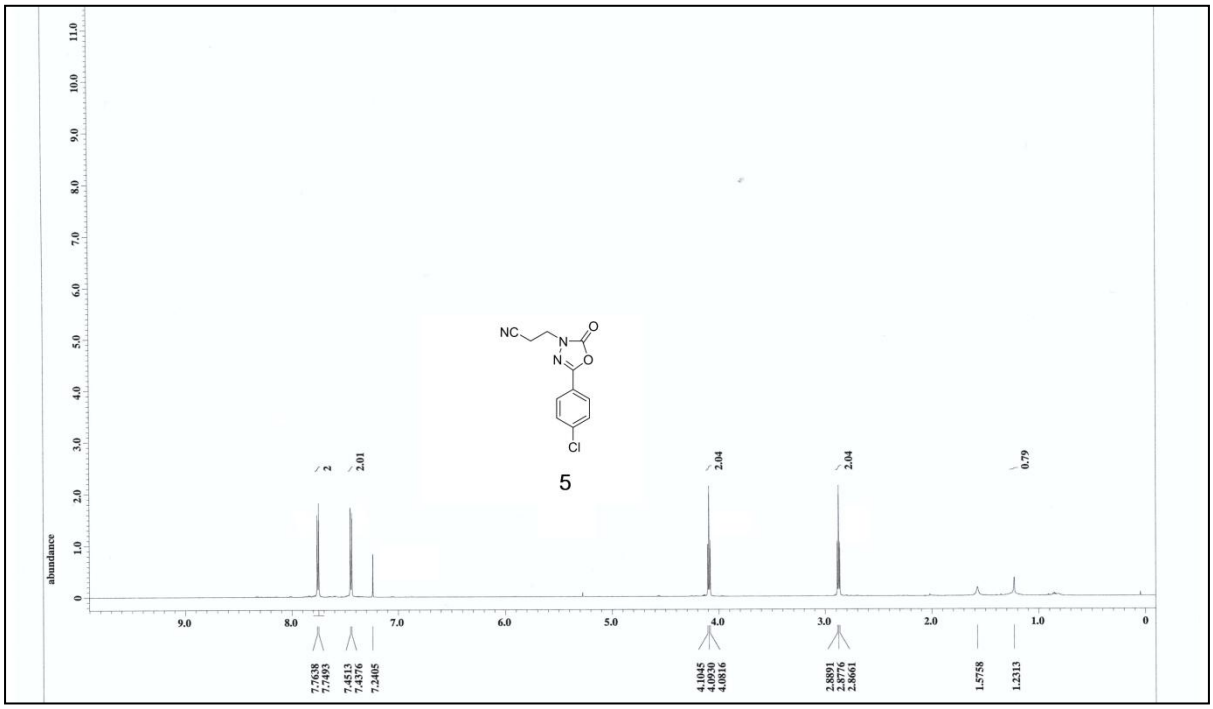
^{1}H NMR of compound **4i** (500 MHz, $CDCl_3$)



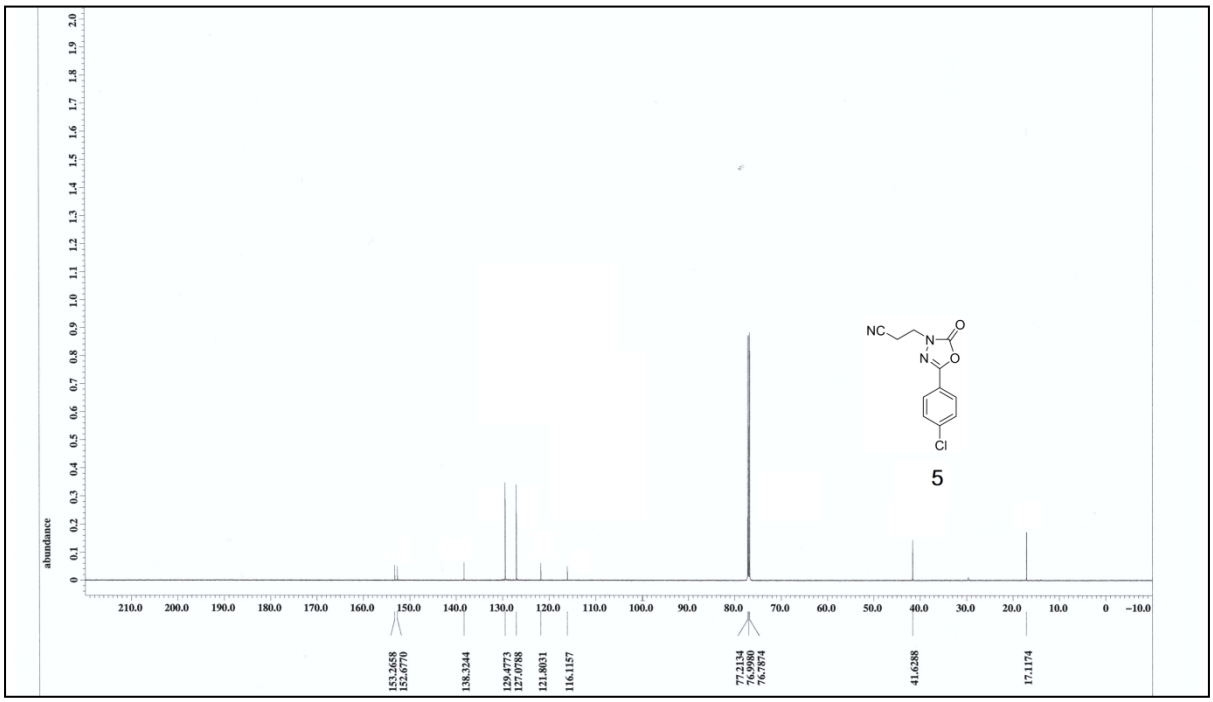
^{13}C NMR of compound **4i** (125 MHz, $CDCl_3$)



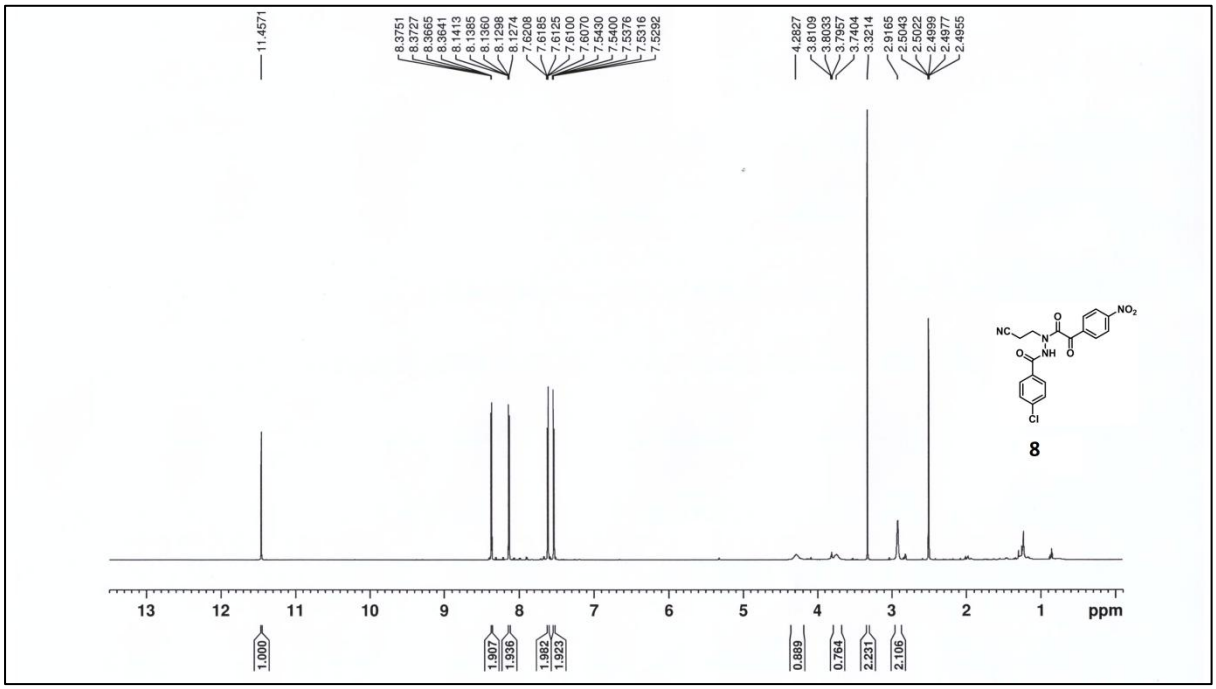
^{1}H NMR of compound **5** (600 MHz, $CDCl_3$)



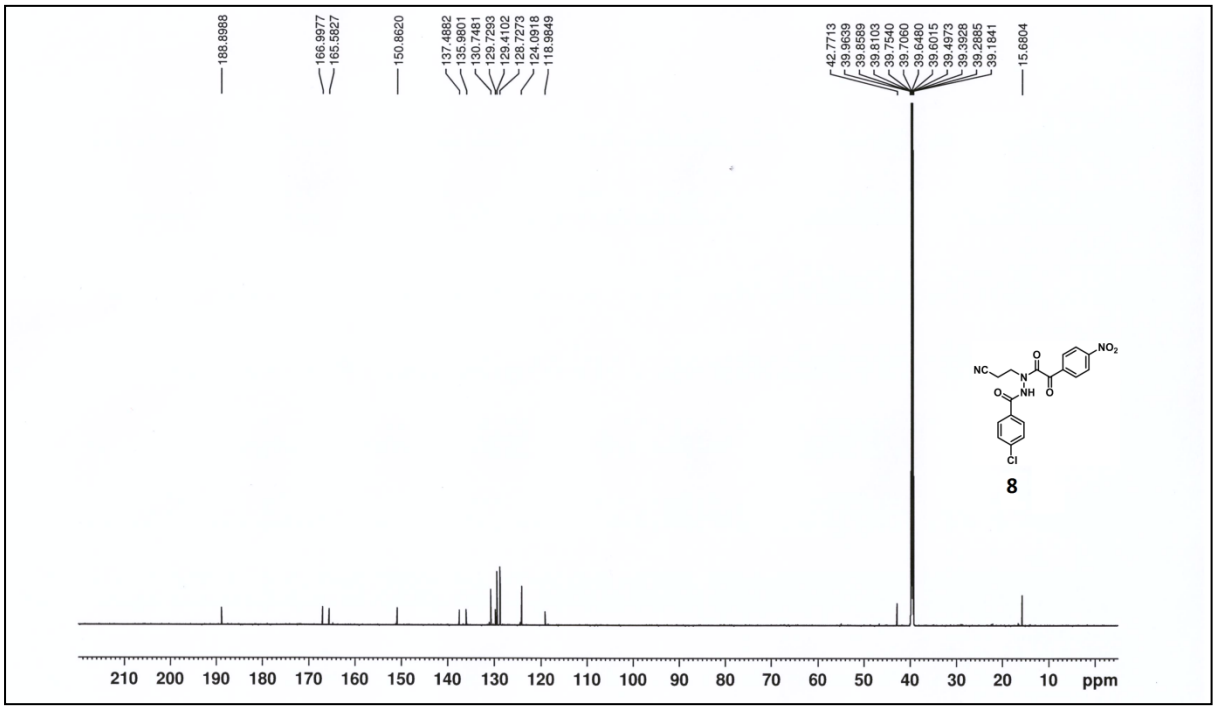
^{13}C NMR of compound **5** (150 MHz, $CDCl_3$)



^{1}H NMR of compound **8** (800 MHz, DMSO-d_6)

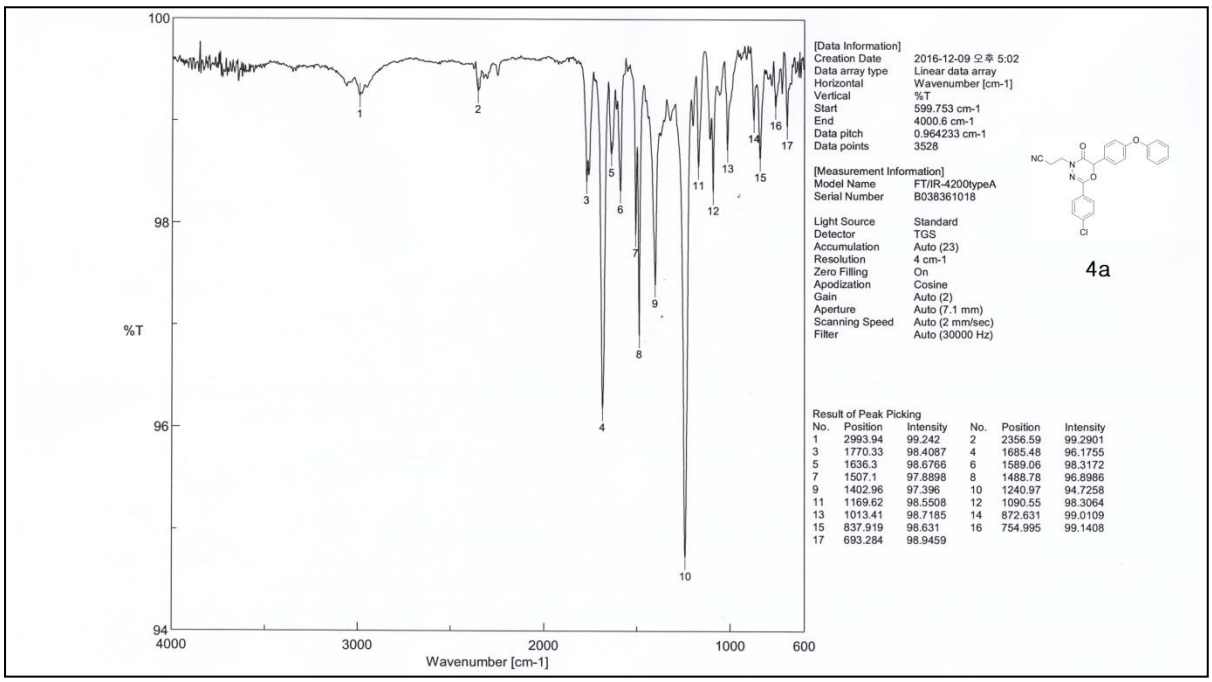


^{13}C NMR of compound **8** (200 MHz, DMSO-d_6)

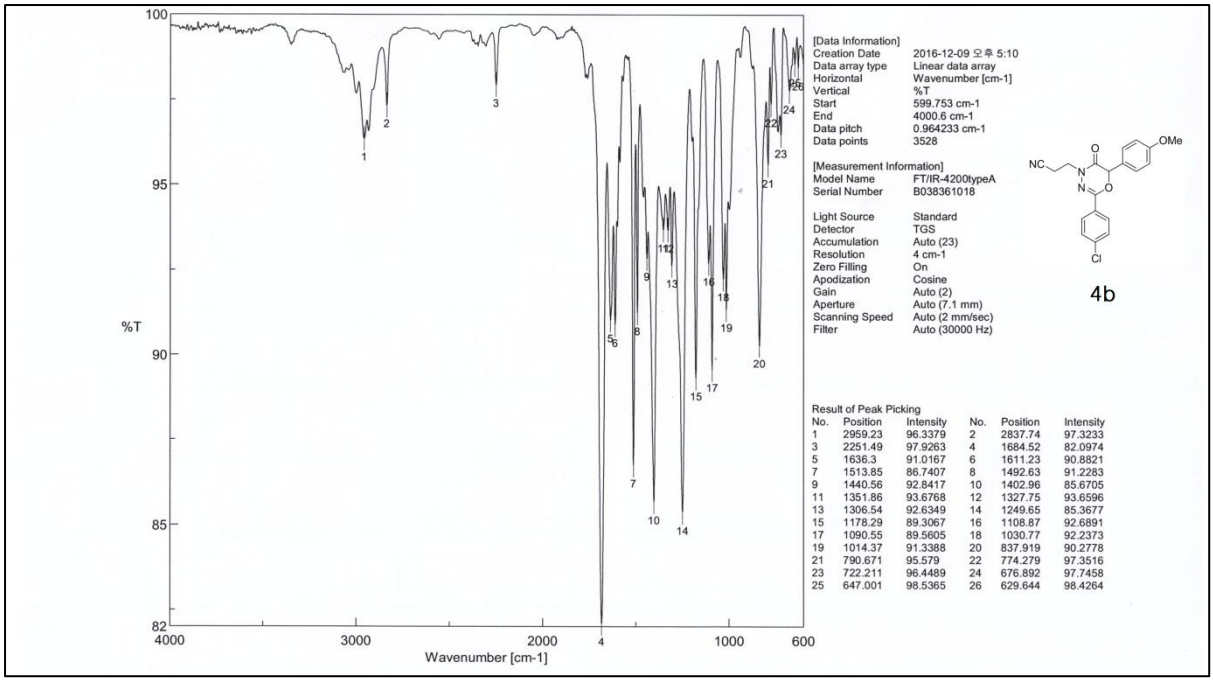


10. [Part 2] IR Spectra

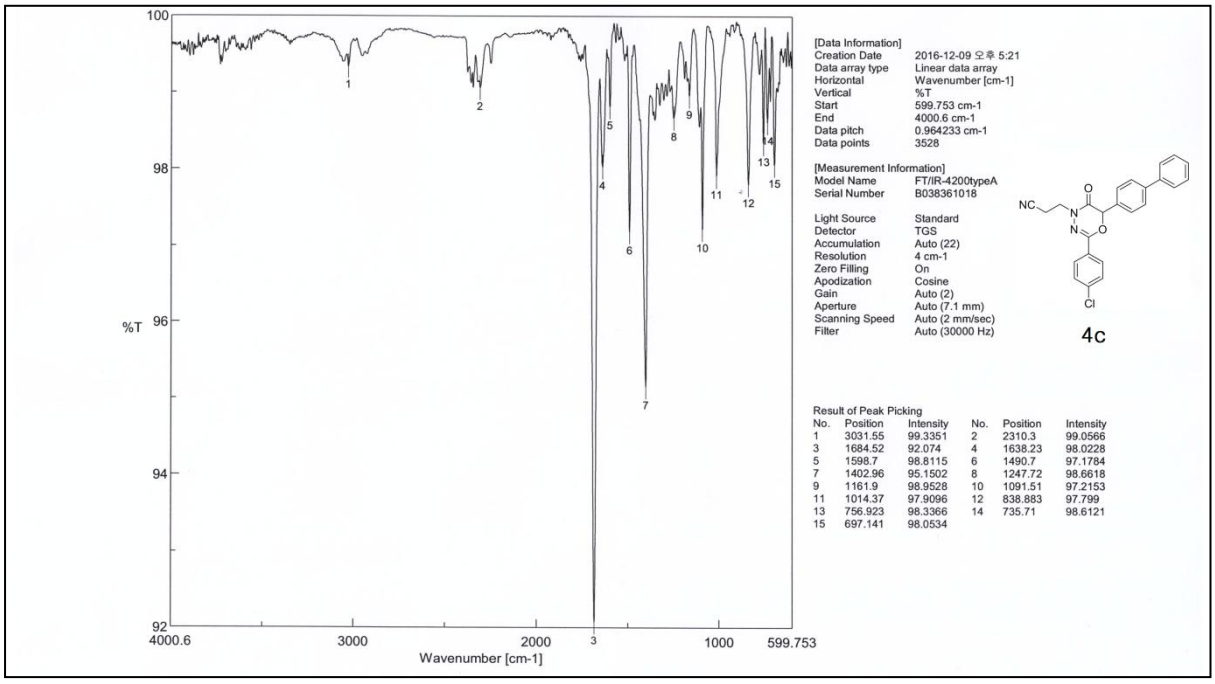
IR Spectrum of **4a**



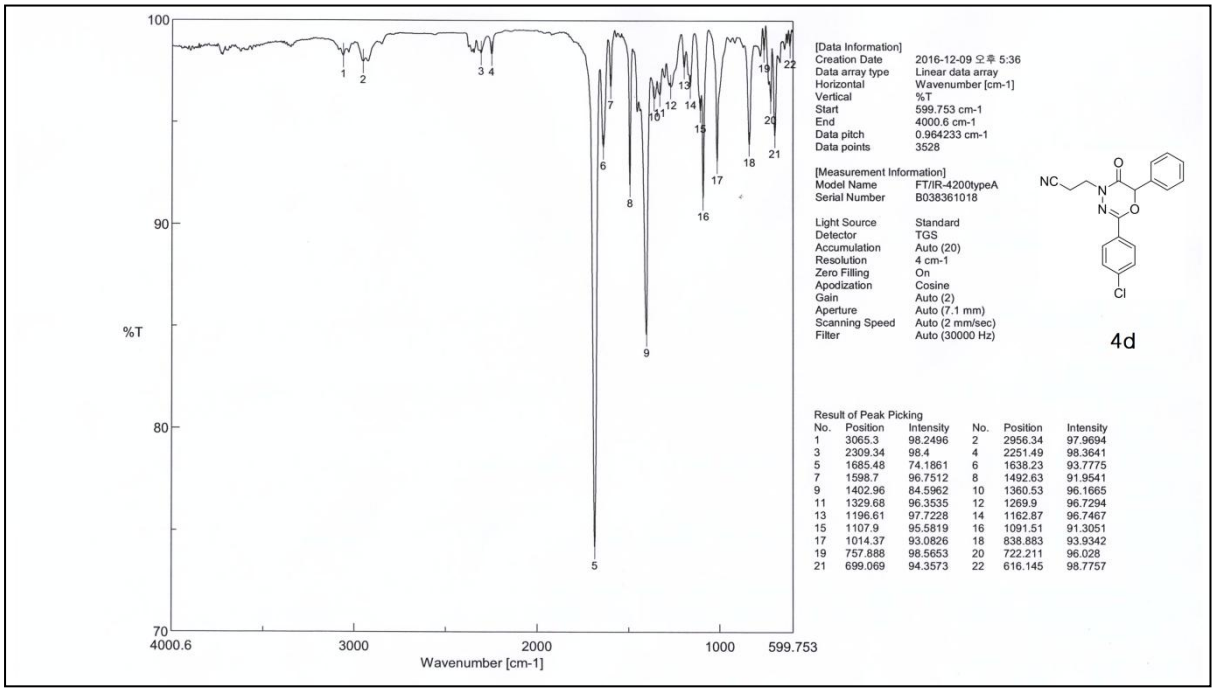
IR Spectrum of **4b**



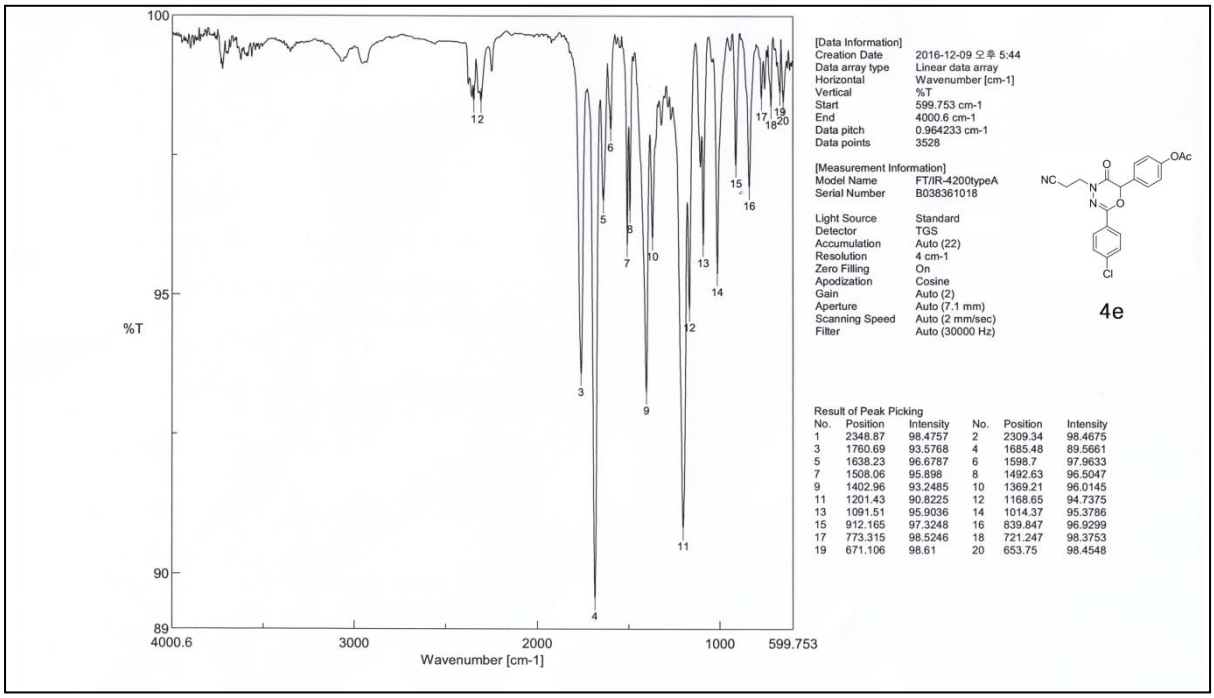
IR Spectrum of **4c**



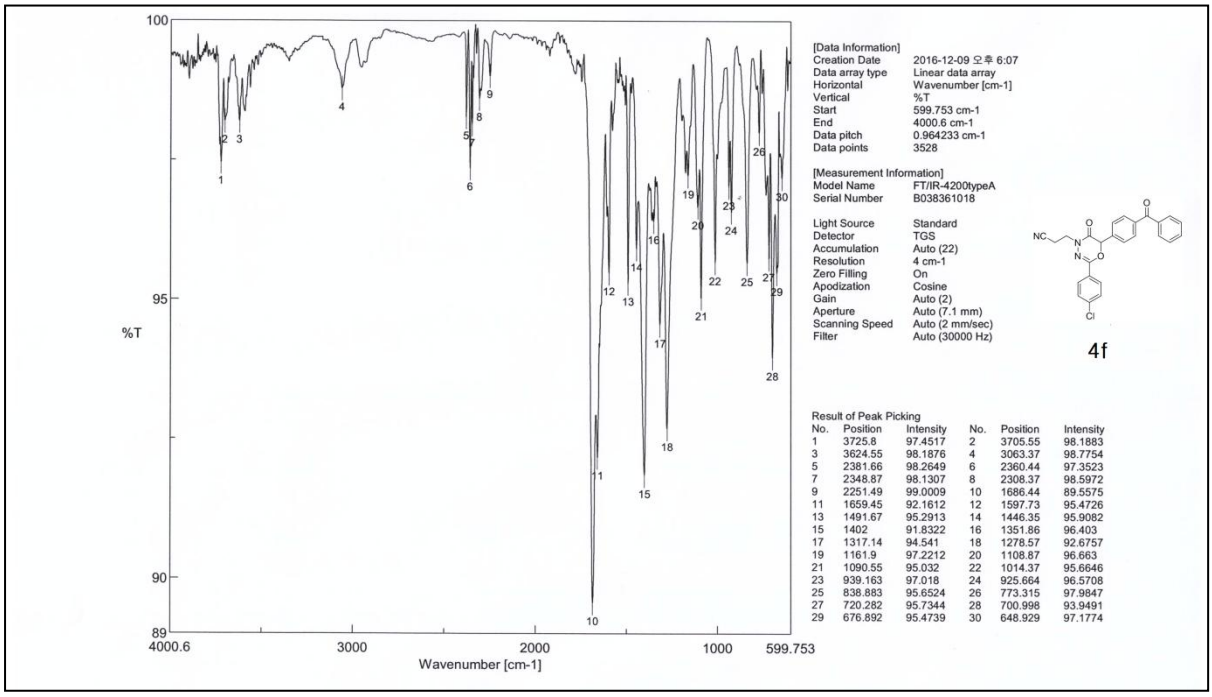
IR Spectrum of **4d**



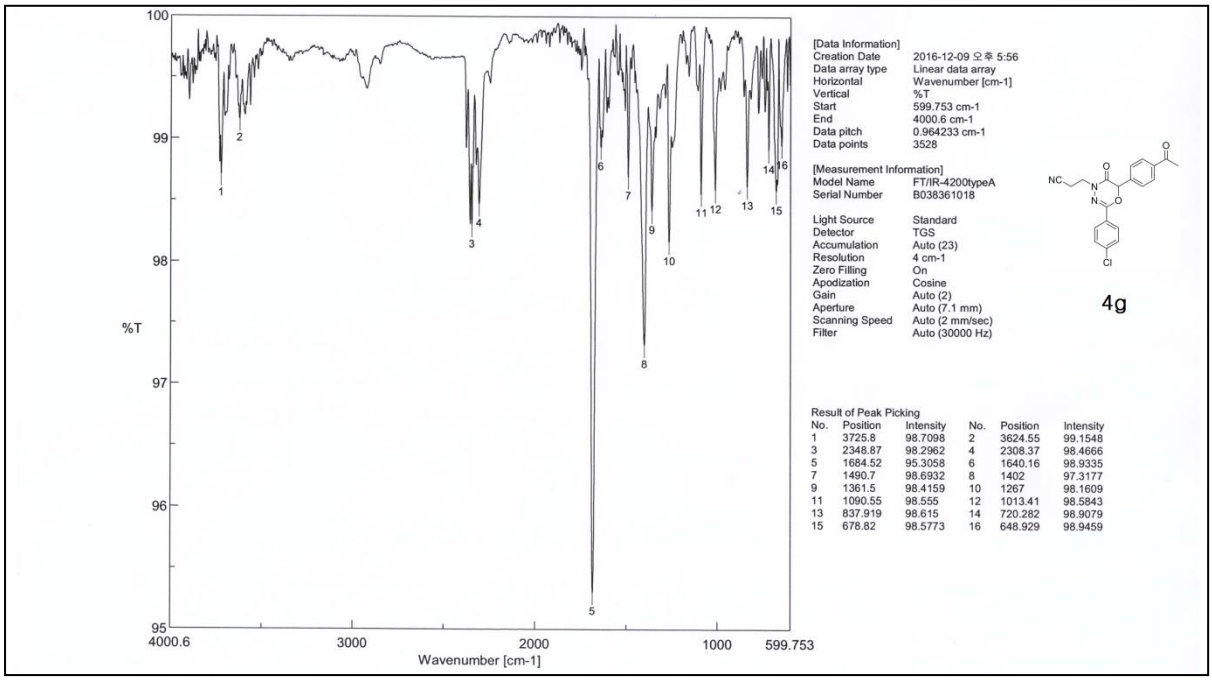
IR Spectrum of **4e**



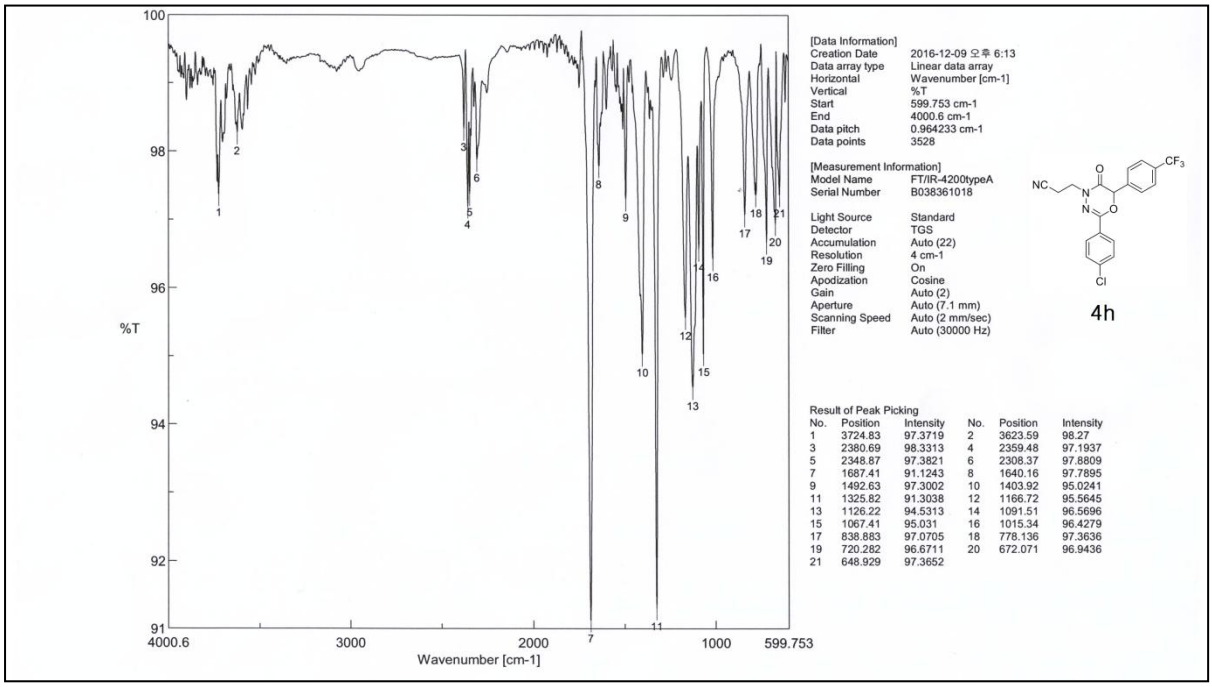
IR Spectrum of **4f**



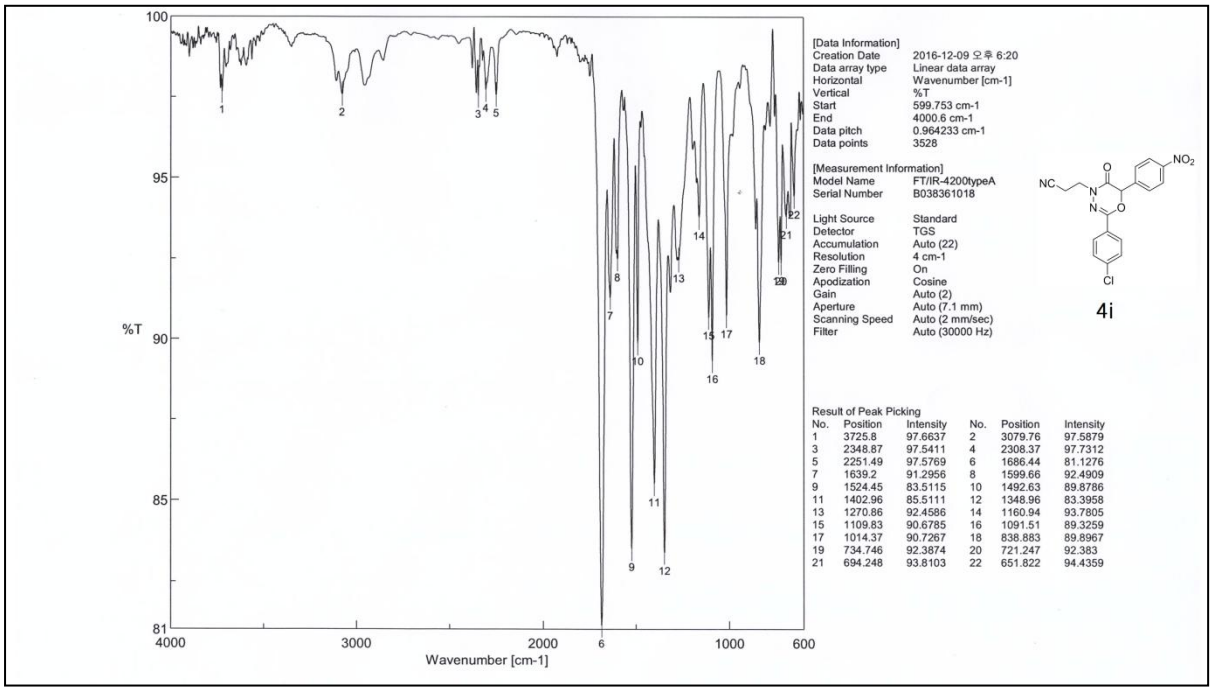
IR Spectrum of **4g**



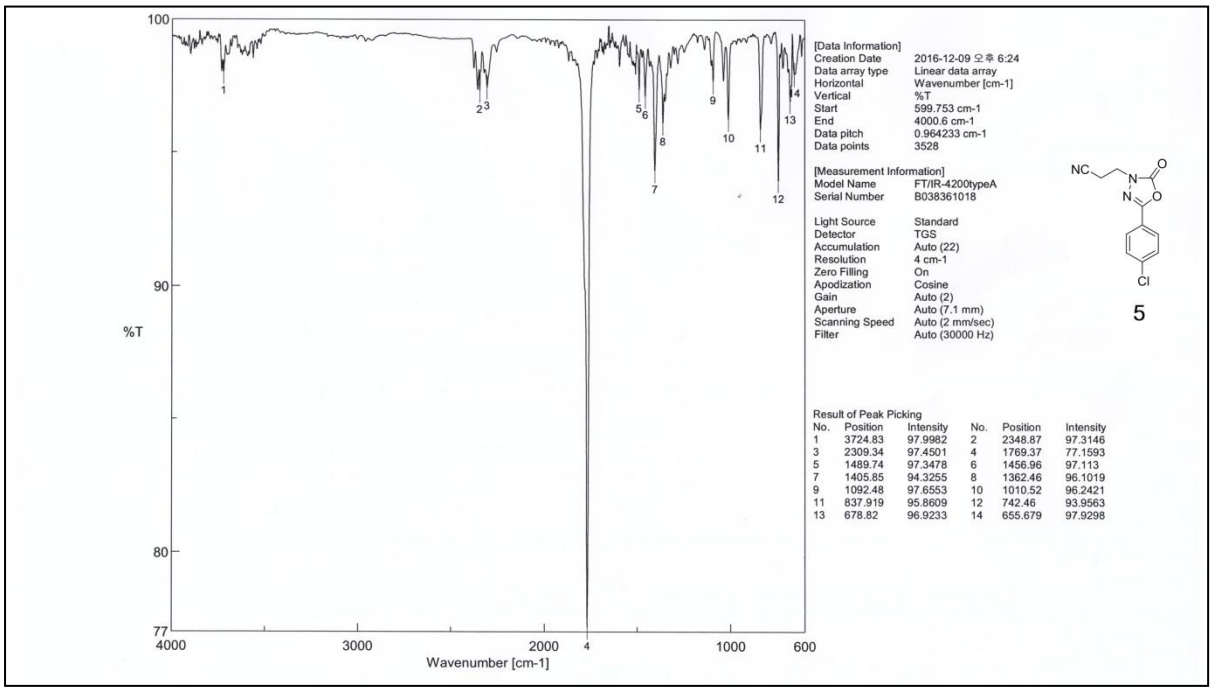
IR Spectrum of **4h**



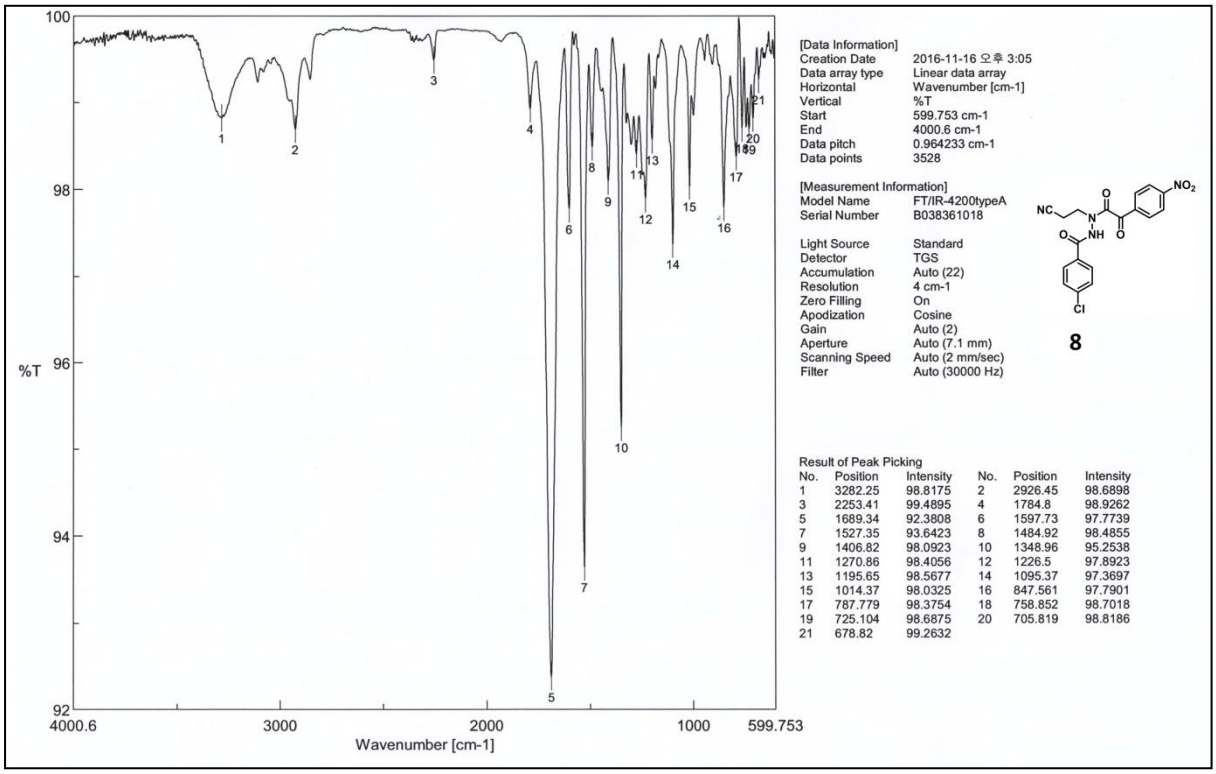
IR Spectrum of **4i**



IR Spectrum of **5**



IR Spectrum of **8**



[Data Information]

Creation Date	2016-11-16 오후 3:05
Data array type	Linear data array
Horizontal	Wavenumber [cm-1]
Vertical	%T
Start	599.753 cm-1
End	4000.6 cm-1
Data pitch	0.964233 cm-1
Data points	3528

[Measurement Information]

Model Name	FT/IR-4200typeA
Serial Number	B038361018
Light Source	Standard
Detector	TGS
Accumulation	Auto (22)
Resolution	4 cm-1
Zero Filling	On
Apodization	Cosine
Gain	Auto (2)
Aperture	Auto (7.1 mm)
Scanning Speed	Auto (2 mm/sec)
Filter	Auto (30000 Hz)

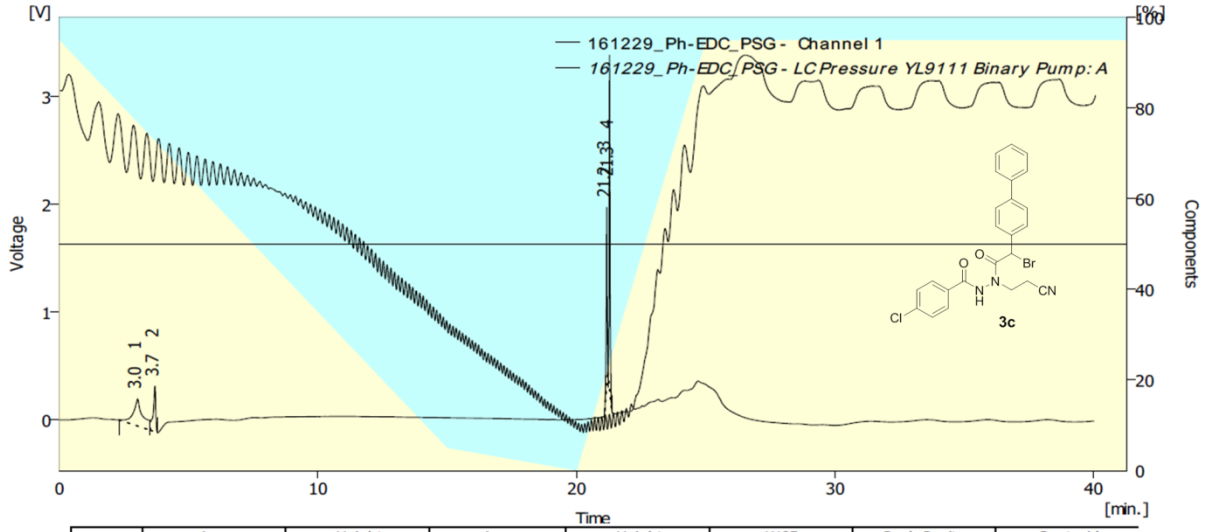
8

Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity
1	3282.25	98.8175	2	2926.45	98.6898
3	2253.41	99.4895	4	1784.8	98.9262
5	1689.34	92.3808	6	1597.73	97.7739
7	1527.35	93.6423	8	1484.92	98.4855
9	1406.82	98.0923	10	1348.96	95.2538
11	1270.86	98.4056	12	1226.5	97.8923
13	1195.65	98.5677	14	1095.37	97.3697
15	1014.37	98.0325	16	847.561	97.7901
17	787.779	98.3754	18	758.852	98.7018
19	725.104	98.6875	20	705.819	98.8186
21	678.82	99.2632			

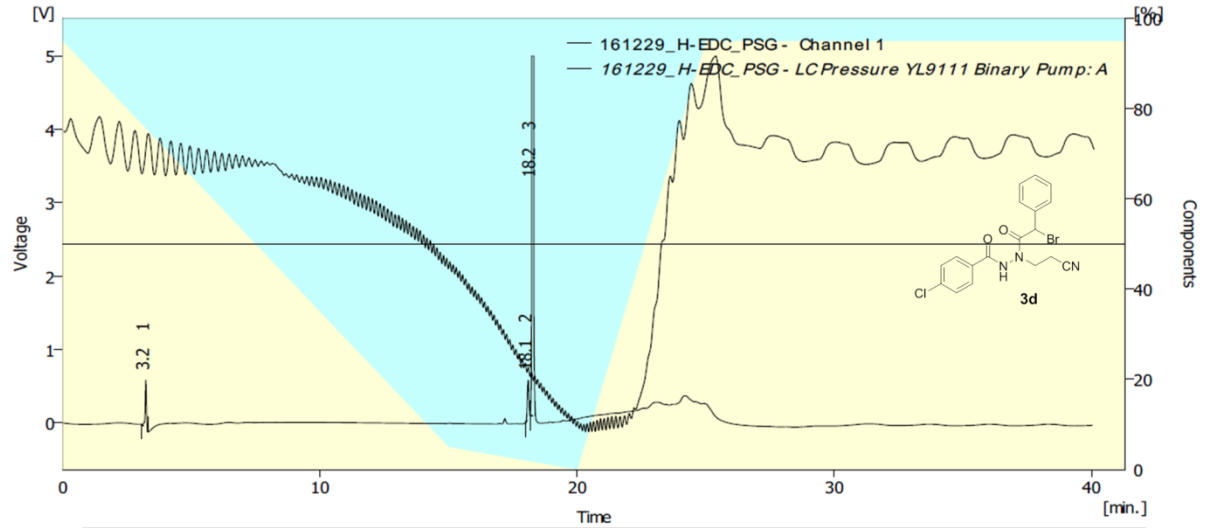
11. [Part 2] HPLC Trace

HPLC trace of **3c**



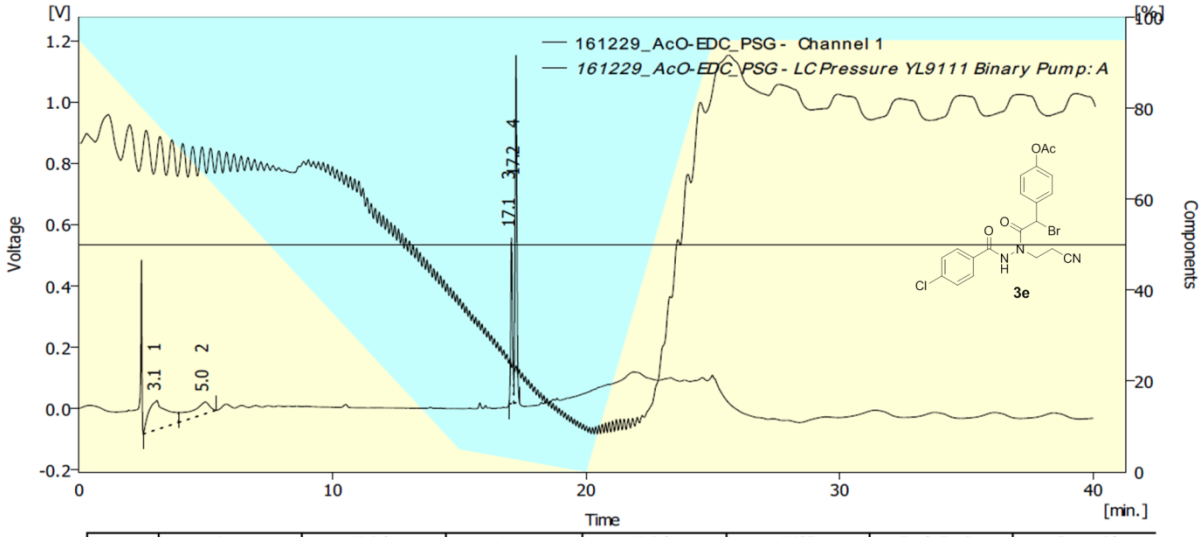
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	6507.884	252.627	31.5	4.7	0.29	483	3.030
2	2817.760	423.949	13.6	7.8	0.09	466	3.710
3	3814.723	1634.307	18.4	30.3	0.03	709	21.167
4	7537.452	3090.368	36.5	57.2	0.03	743	21.280
	20677.819	5401.252	100.0	100.0			

HPLC trace of **3d**



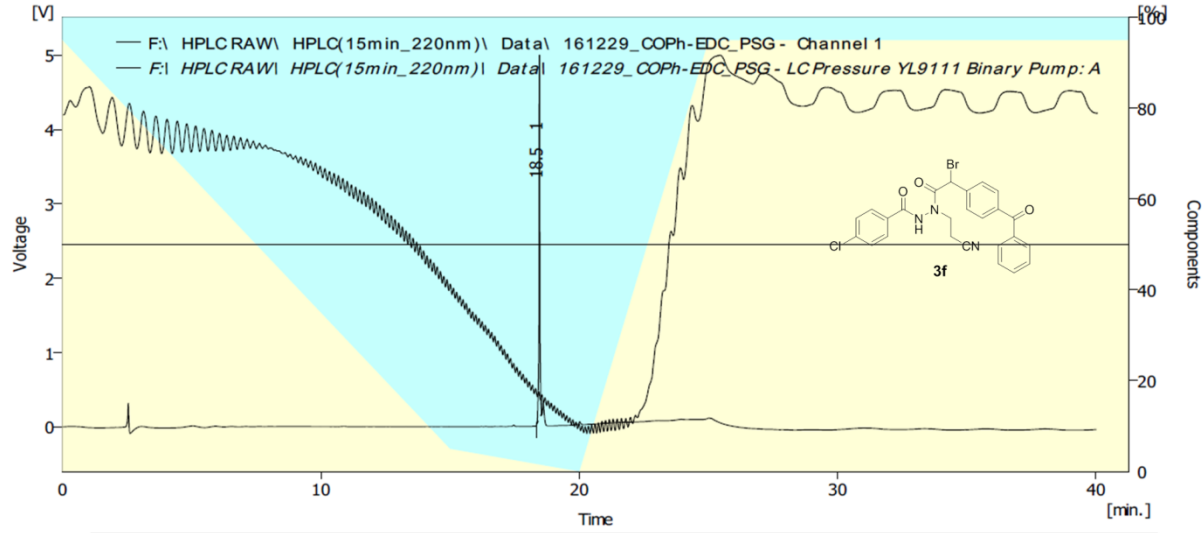
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	2295.023	666.612	7.0	11.0	0.05	409	3.214
2	2254.394	519.401	6.9	8.5	0.07	719	18.086
3	28104.375	4899.279	86.1	80.5	0.09	740	18.261
	32653.792	6085.292	100.0	100.0			

HPLC trace of **3e**



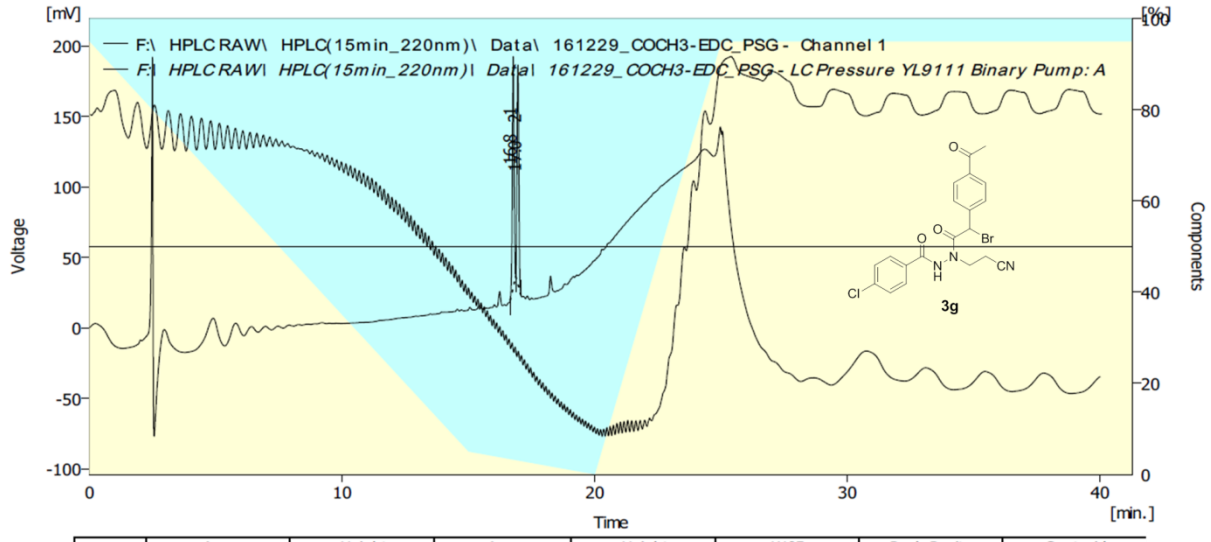
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	4803.349	94.653	31.8	5.2	1.22	616	3.191
2	2226.609	39.397	14.7	2.2	0.53	588	5.056
3	2624.015	541.026	17.4	29.9	0.08	647	17.063
4	5466.597	1134.246	36.2	62.7	0.08	577	17.224
	15120.570	1809.322	100.0	100.0			

HPLC trace of **3f**



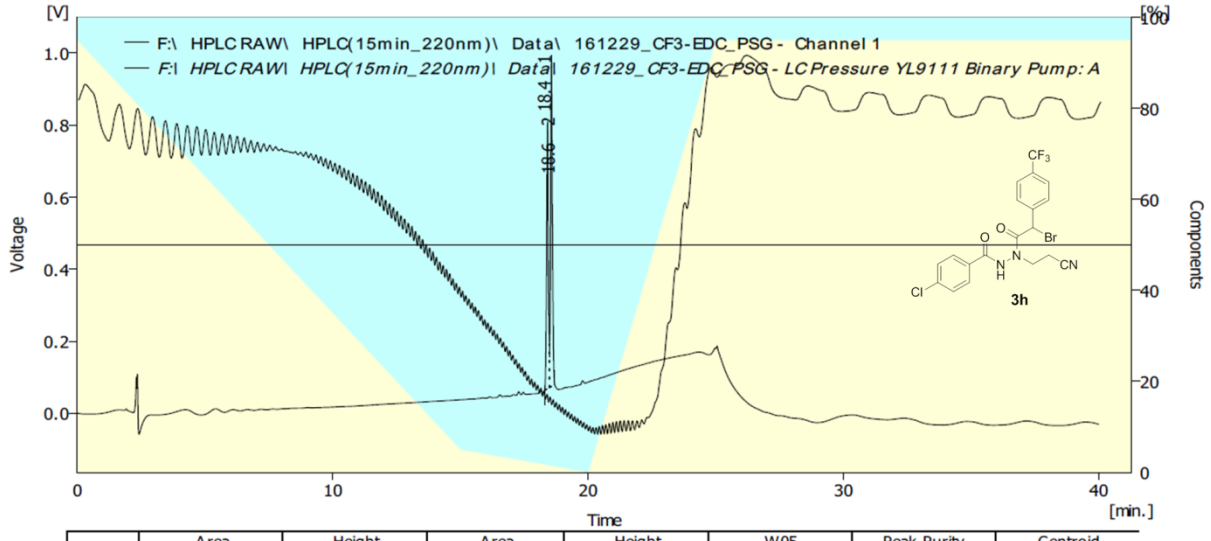
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	12513.582	4889.630	100.0	100.0	0.04	674	18.459
	12513.582	4889.630	100.0	100.0			

HPLC trace of **3g**



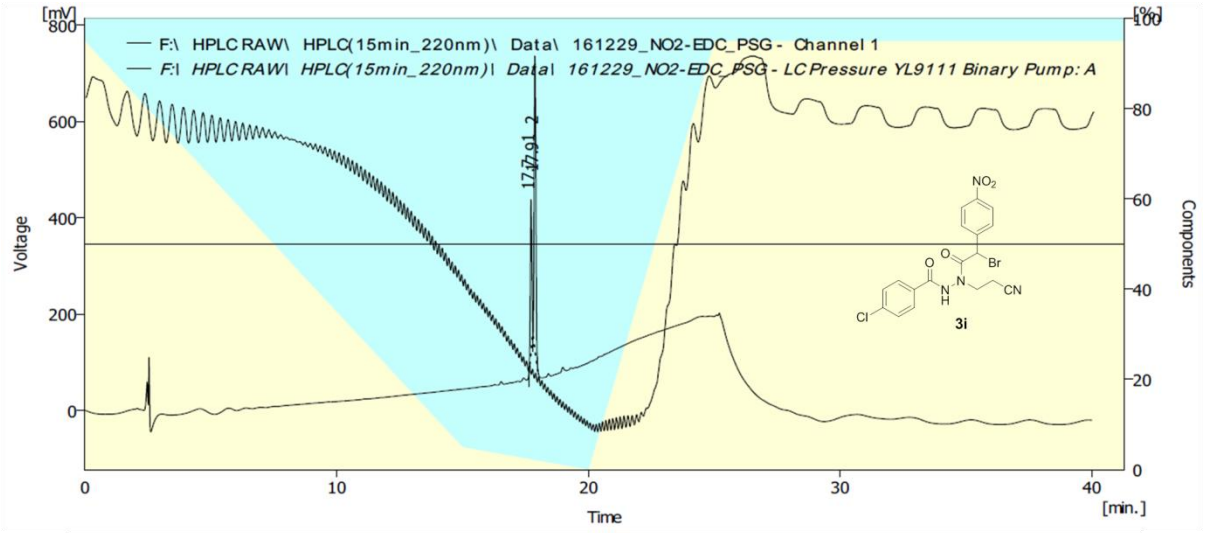
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	770.249	163.995	49.6	51.3	0.08	606	16.769
2	784.169	155.545	50.4	48.7	0.09	655	16.958
	1554.419	319.541	100.0	100.0			

HPLC trace of **3h**



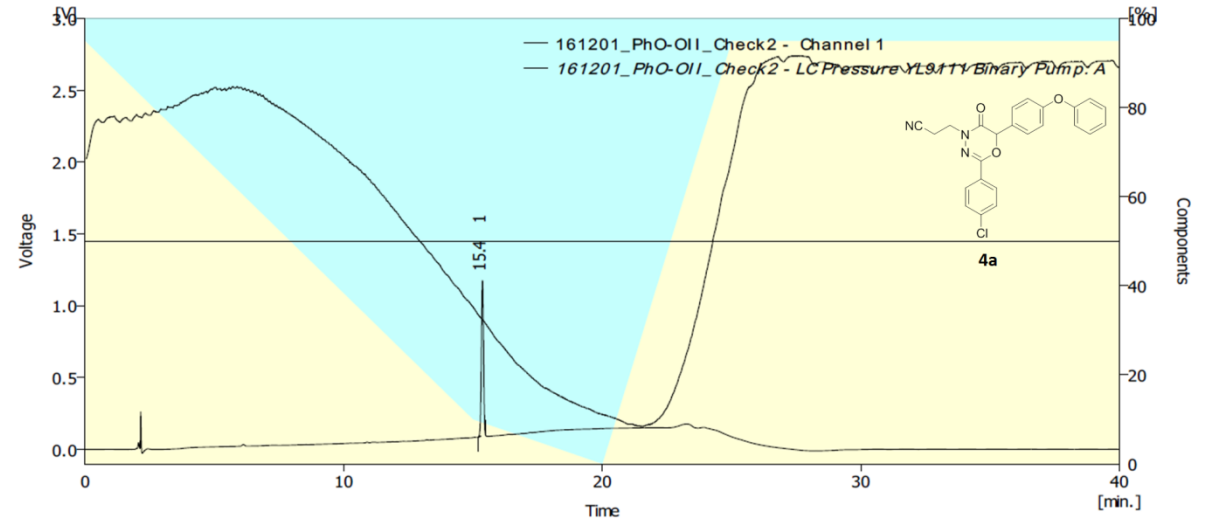
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	3483.919	740.144	44.1	44.6	0.07	739	18.408
2	4415.225	919.241	55.9	55.4	0.08	742	18.564
	7899.145	1659.385	100.0	100.0			

HPLC trace of **3i**



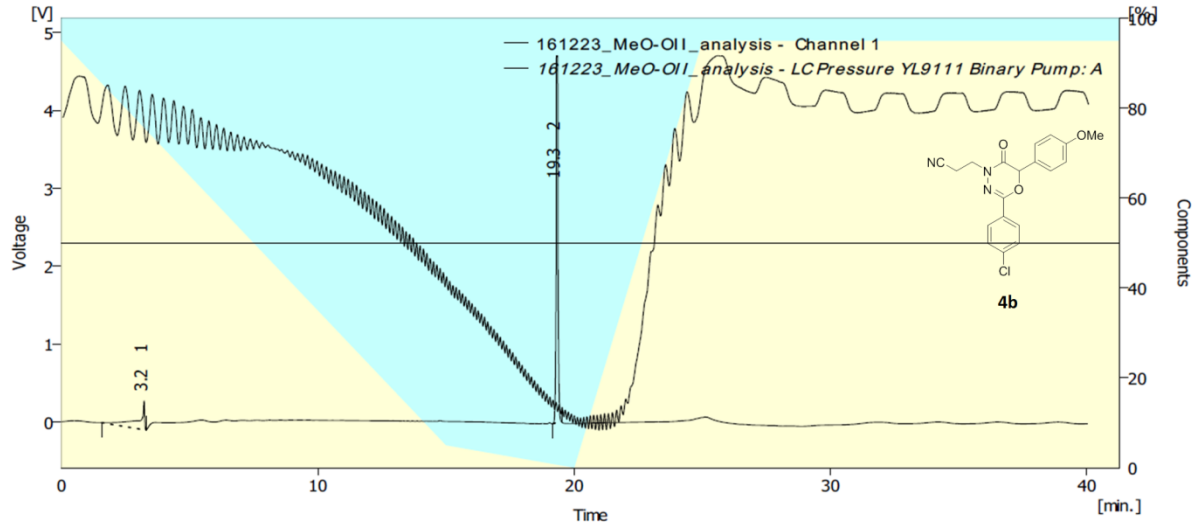
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	1291.861	314.849	30.8	34.0	0.07	829	17.719
2	2902.438	609.846	69.2	66.0	0.08	795	17.881
	4194.299	924.695	100.0	100.0			

HPLC trace of **4a**



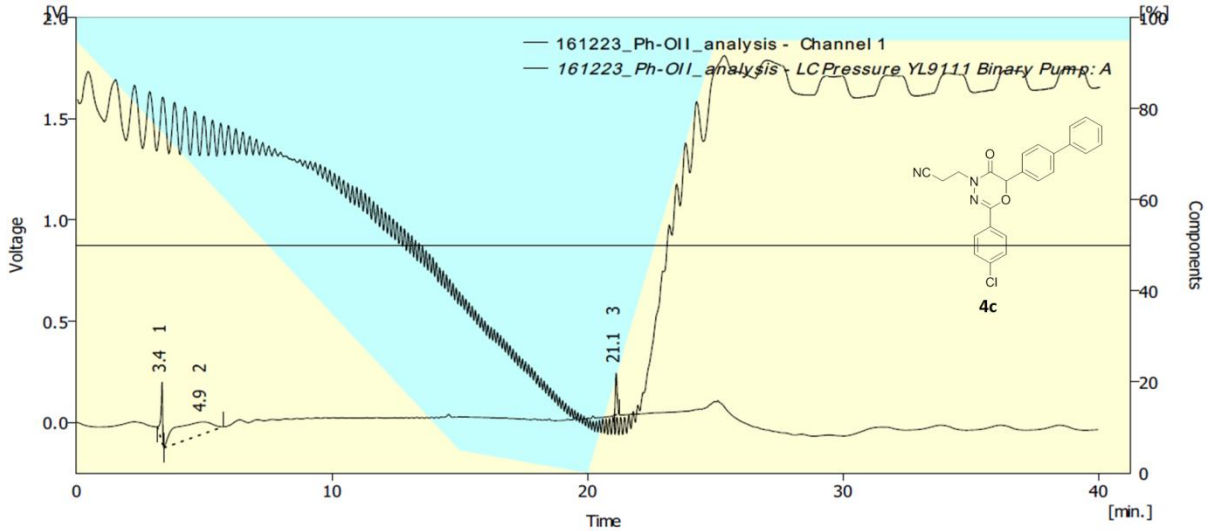
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	6245.369	1080.332	100.0	100.0	0.09	688	15.352
	6245.369	1080.332	100.0	100.0			

HPLC trace of **4b**



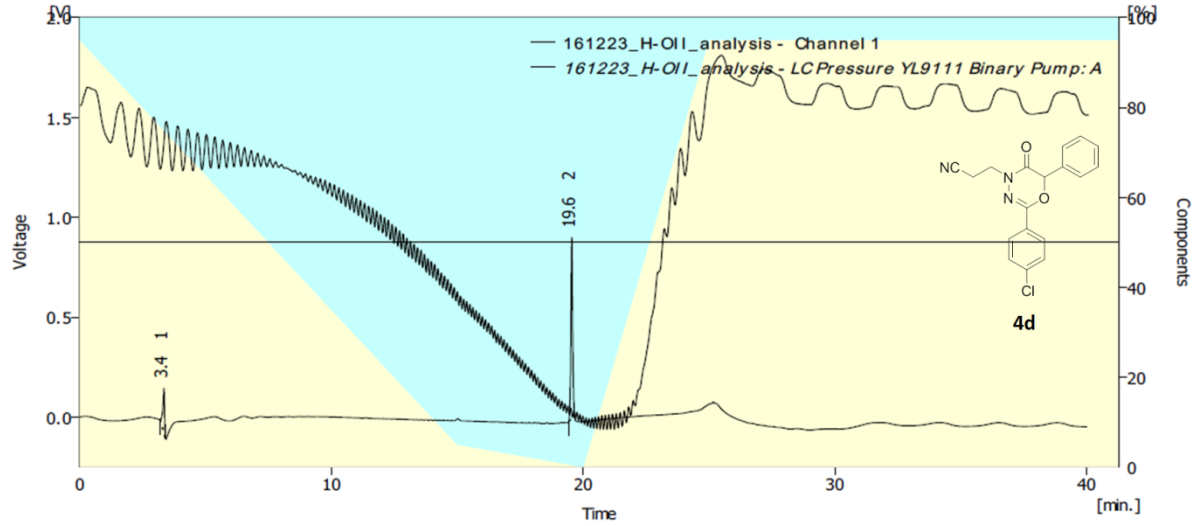
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	6699.755	372.540	21.2	7.3	0.06	764	3.632
2	24877.312	4716.138	78.8	92.7	0.07	600	19.330
	31577.067	5088.677	100.0	100.0			

HPLC trace of **4c**



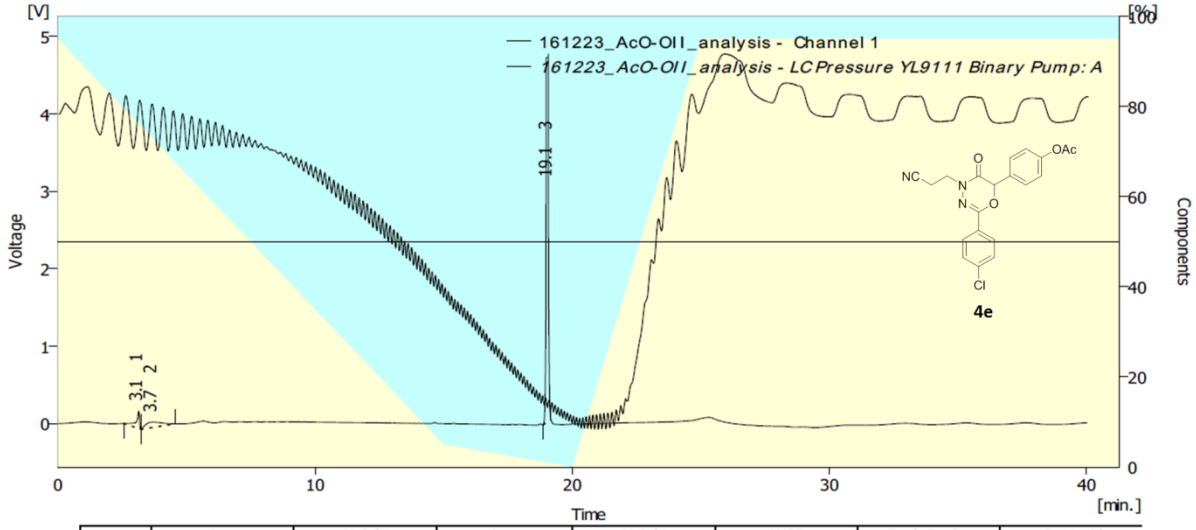
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	1333.289	295.546	13.7	52.7	0.06	541	3.361
2	7420.342	60.034	76.2	10.7	1.93	722	4.974
3	982.071	205.292	10.1	36.6	0.08	625	21.136
	9735.701	560.871	100.0	100.0			

HPLC trace of **4d**



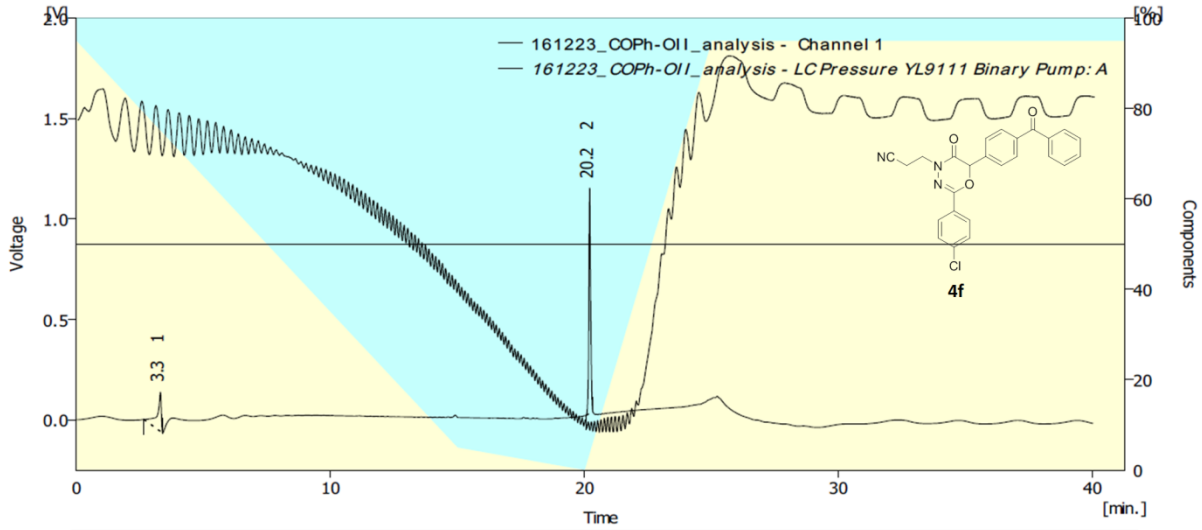
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	1044.717	224.586	19.6	19.8	0.08	490	3.358
2	4286.179	909.445	80.4	80.2	0.07	728	19.551
	5330.896	1134.031	100.0	100.0			

HPLC trace of **4e**



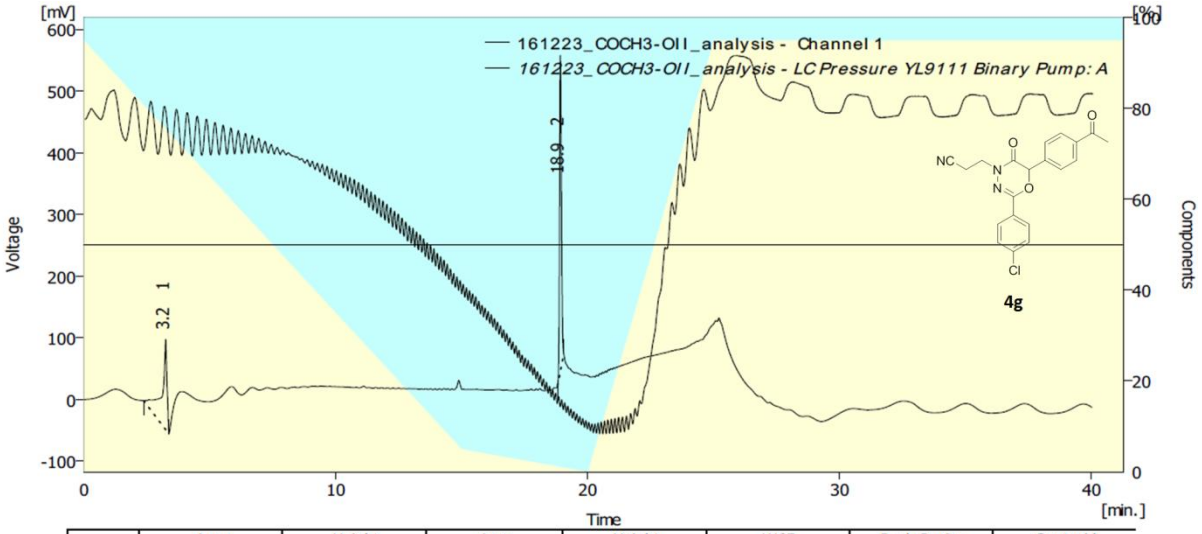
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	2156.523	228.756	5.9	4.5	0.09	690	3.254
2	3097.552	68.052	8.4	1.3	1.17	864	3.811
3	31414.190	4747.490	85.7	94.1	0.10	600	19.080
	36668.265	5044.298	100.0	100.0			

HPLC trace of **4f**



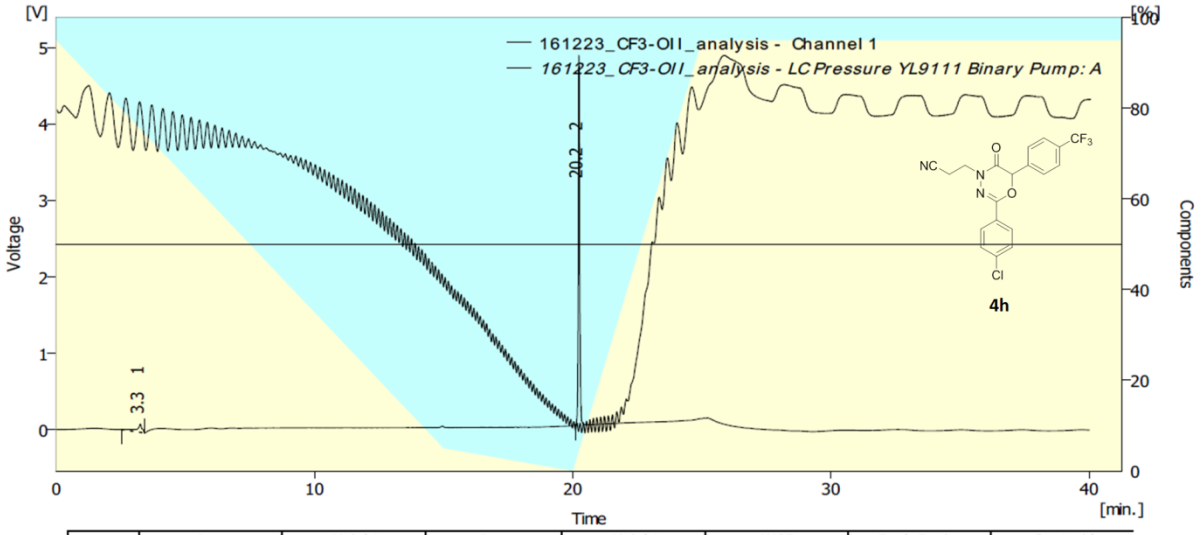
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	2256.317	195.580	29.8	14.8	0.12	612	3.420
2	5310.431	1122.679	70.2	85.2	0.07	635	20.207
	7566.748	1318.259	100.0	100.0			

HPLC trace of **4g**



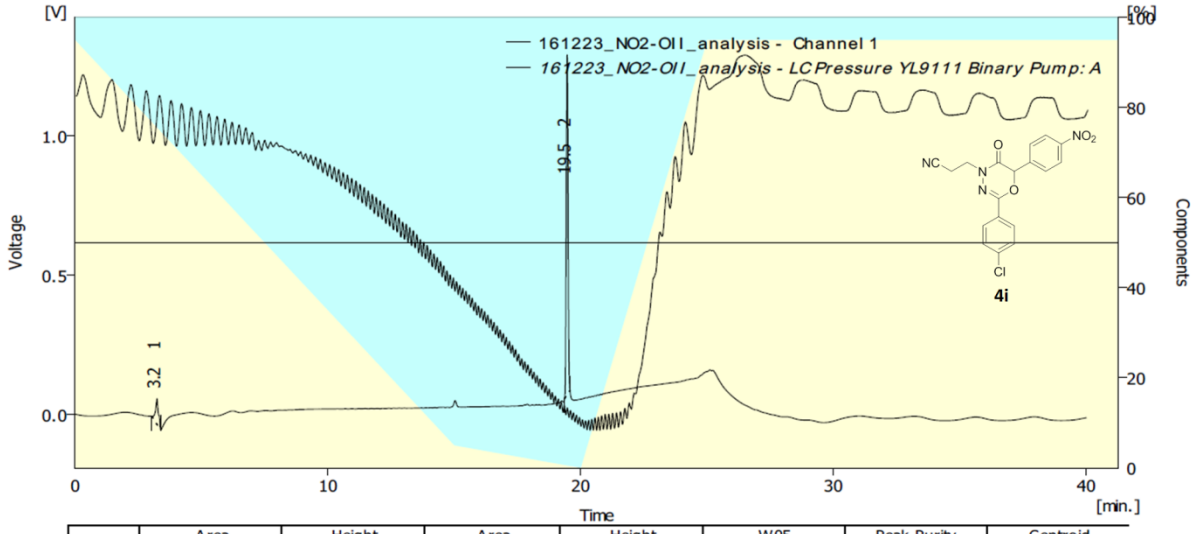
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	2172.197	146.427	45.3	22.2	0.13	294	3.395
2	2623.020	512.166	54.7	77.8	0.09	597	18.922
	4795.217	658.592	100.0	100.0			

HPLC trace of **4h**



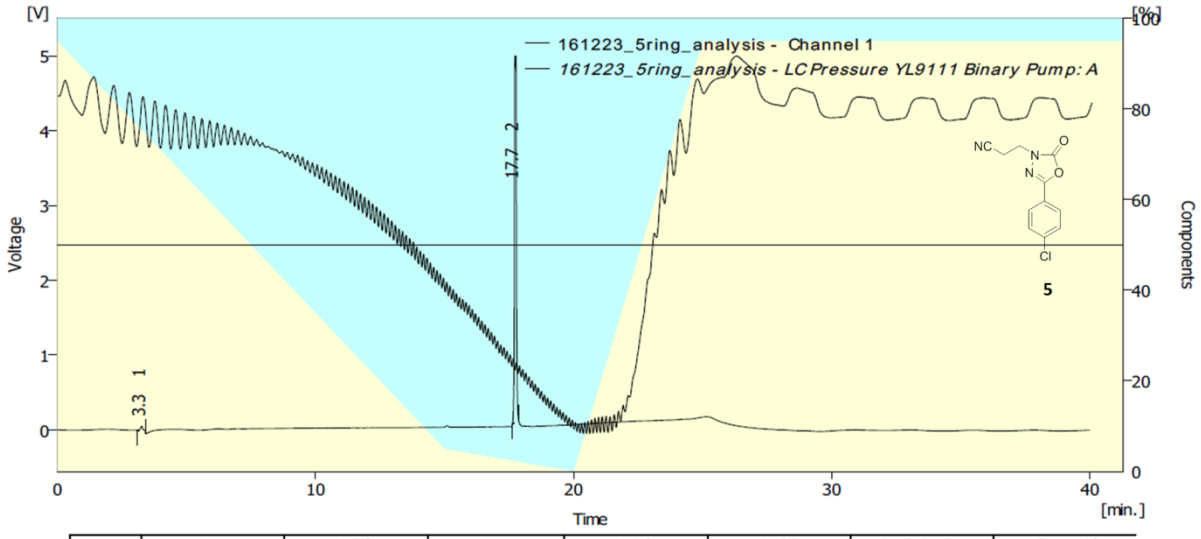
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	1556.379	109.207	8.1	2.2	0.13	451	3.337
2	17545.047	4811.642	91.9	97.8	0.05	570	20.248
	19101.425	4920.849	100.0	100.0			

HPLC trace of **4i**



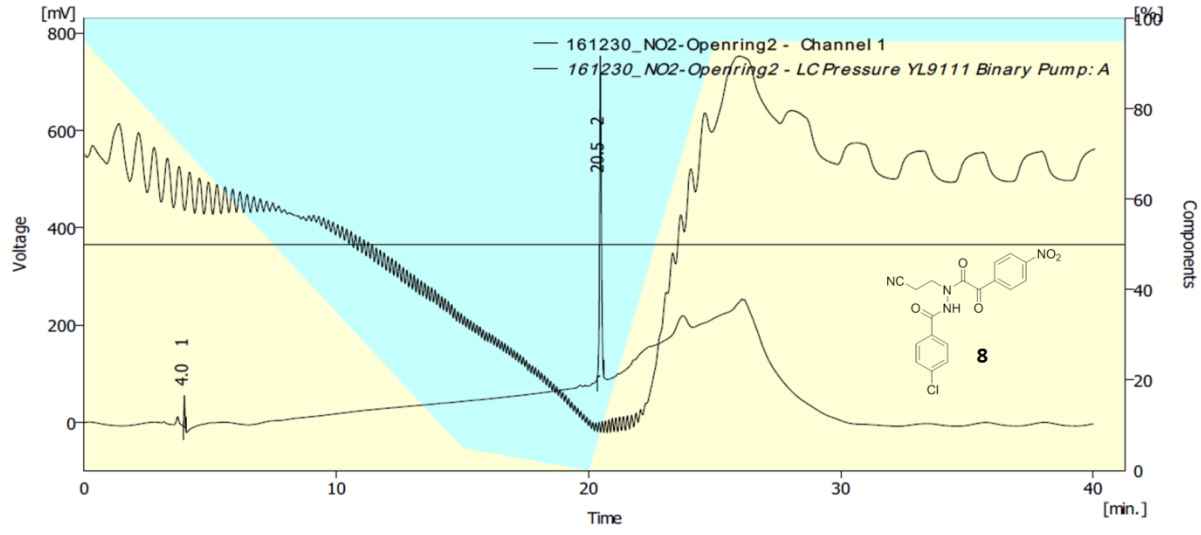
	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	761.516	92.527	11.1	7.0	0.11	482	3.215
2	6075.315	1220.855	88.9	93.0	0.08	665	19.468
	6836.831	1313.382	100.0	100.0			

HPLC trace of **5**



	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	703.580	77.380	2.6	1.6	0.11	608	3.266
2	26066.911	4899.045	97.4	98.4	0.08	765	17.739
	26770.491	4976.425	100.0	100.0			

HPLC trace of **8**



	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Centroid [min]
1	182.764	67.124	5.4	9.3	0.05	701	3.975
2	3182.195	657.248	94.6	90.7	0.08	746	20.472
	3364.959	724.372	100.0	100.0			

Abstracts

[Part 1]

Design of the 1,3,4-oxadiazin-5(6*H*)-one derivatives with antitumor activities using molecular modeling

Seunggun Park

College of Pharmacy

The Graduate School

Seoul National University

The 1,3,4-oxadiazin-5(6*H*)-one is a peptidomimetic compounds which are N,O-containing heterocyclic scaffolds. Previous studies by other groups has been reported 1,3,4-oxadiazin-5(6*H*)-one derivatives of the N4 or C2 positions for synthesis or several biological effects. Recently, our group reported the 1,3,4-oxadiazin-5(6*H*)-one derivatives functionalized at the C6 position for anticancer activity.

In our continuous efforts to comprehend the biological effects about the functionality of the 1,3,4-oxadiazin-5(6*H*)-one scaffold, we synthesized new 1,3,4-oxadiazin-5(6*H*)-one derivatives with various alkyl and aryl groups at C6 position. The antitumor activities of 1,3,4-oxadiazin-5(6*H*)-one derivatives was screened by using MTT assay and we designed the scaffold containing expanded functionality using molecular modeling. The Strategy explored in this report will provide valuable tools to expand the functionality of N,O-containing heterocyclic scaffolds for improvement of the biological activities.

Keyword: 1,3,4-oxadiazin-5(6*H*)-one, functionalization, molecular modeling

Student Number: 2015-21875

[Part 2]

Study on synthesis and substituent effects of 1,3,4-oxadiazin-5(*6H*)-one derivatives functionalized at the C6 position

Seunggun Park

College of Pharmacy

The Graduate School

Seoul National University

The 1,3,4-oxadiazin-5(6*H*)-one is one of the N,O-containing heterocyclic scaffolds with some biological effects reported including preliminary antitumor and neurological therapeutic activities. Previous reports by other groups have used derivatization of 1,3,4-oxadiazin-5(6*H*)-one only at the N4 or C2 positions. Recently, our group reported the 1,3,4-oxadiazin-5(6*H*)-one derivatives functionalized at the C6 position for anticancer activity.

In our continuous efforts to expand the functionality of the 1,3,4-oxadiazin-5(6*H*)-one scaffold, we synthesized new 1,3,4-oxadiazin-5(6*H*)-one derivatives with p-substituted phenyl groups at C6 position. EDC coupling of arylhydrizides followed by a cyclization reaction using a base led to the derivatives of 1,3,4-oxadiazin-5(6*H*)-one in good yields. The cyclization reaction was affected by the electronic effect of *p*-substituted phenyl group under various conditions. Synthetic strategy explored in this report will provide valuable tools to expand the functionality of N,O-containing heterocyclic scaffolds.

Keyword: 1,3,4-oxadiazin-5(6*H*)-one, Cyclization, Substituent, electronic effect

Student Number: 2015-21875